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HIDROCARBUROS CUTICULARES: SU POTENCIAL COMO ESTÍMULO PARA EL RECONOCIMIENTO DE PARENTELA EN DOS ARAÑAS SUBSOCIALES *ANELOSIMUS* (ARANEAE: THERIDIIDAE) Y PATRONES DE FIJACIÓN DE HILOS DE SEDA EN LA ARAÑA SUBSOCIAL *ANELOSIMUS BAEZA* (ARANEAE: THERIDIIDAE)

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Dedicatoria

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RESUMEN

El reconocimiento de parentela en grupos sociales de artrópodos puede ocurrir por diferentes tipos de estímulos, como por ejemplo los compuestos excretados de la cutícula. Un rasgo posiblemente ancestral de las arañas es organizar los comportamientos en modulos. Se estudió dos especies de arañas del género *Anelosimus* (Theridiidae). Las cuales tienen diferente nivel de socialidad y construyen una tela poco estudiada en forma de canasta que consiste de una plancha debajo de una maraña. Primero se investigó si los compuestos cuticulares de dos especies (*Anelosimus baeza* y *Anelosimus* cf. *studiosus*) podrían tener potencial como estímulo para el reconocimiento de parentela. Segundo se estudiaron los patrones de comportamiento de fijación y manipulación de hilos de seda por la araña *A. baeza*. Estos patrones están bien conservados a través de diferentes grupos taxonómicos.

Se estudió si los compuestos cuticulares podían funcionar como estímulo para la discriminación de especies y parentela en dos especies subsociales que viven en colonias. Existe muy poca información sobre compuestos de cutícula en arañas con algún nivel de socialidad y hasta la fecha no existe una comparación entre especies. La mayoría de trabajos se han realizado en insectos eusociales. Se colectaron arañas de *A. baeza* y *A.* cf. *studiosus* y se extrajo sus compuestos de cutícula. Se encontró que los perfiles químicos de las especies son distintos y que los las colonias también tienen perfiles químicos característicos. También se encontró que ambas especies comparten un número pequeño de compuestos químicos y comparten también otros compuestos con otras especies de arañas sociales, lo que indicaría que sí hay potencial para ser utilizados en reconocimiento de parentela. Se observó que *A.* cf. *studiosus* tiene menos compuestos pesados en su cutícula que *A. baeza*. Arañas con bajo nivel de socialidad como *A.* cf. *studiosus* tienden a ser menos tolerantes hacia familiares cuando sus cutículas tienen más compuestos pesados. Lo que sugiere que en especies más sociales como *A. baeza* los compuestos pesados no son un factor que afecte la tolerancia.

Adicionalmente se probó que algunos detalles de fijación de hilos están distribuídos ampliamente entre araneoidos, incluídos tejedores de tela orbiculares y teridios. Se encontró que *A. baeza* presenta similitudes con ambos grupos lo que indica que el diseño de la tela no influye en los patrones de fijación. *A. baeza* mostró flexibilidad en los comportamientos de fijación y destreza en movimientos estereotipados una necesidad para arañas orbiculares a la hora de construir sus telas.

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INTRODUCCIÓN GENERAL

Comportamiento social, eusocialidad y las otras sociedades animales. El comportamiento social es aquel que resulta de la interacción de dos o más individuos y no puede ocurrir individualmente. Se cree que la evolución de los sistemas sociales en vertebrados e invertebrados ocurrió como respuesta a una combinación de restricciones ecológicas que limitaban la capacidad de supervivencia de individuos solitarios (Rypstra 1993).

Existen diferentes grados de interacción entre especies y por ende diferentes grados de socialidad, y por esta razón surge la necesidad de clasificar dichos comportamientos. La clasificación más sencilla de jerarquía (de menor a mayor nivel social) sería la de Costa (2006): 1- subsocial: donde hay cuido parental de inmaduros; 2- comunal: donde miembros de la misma generación viven juntos pero no cuidan a la progenie; 3- quasisocial: miembros de la misma generación viven juntos y cuidan a la progenie; 4- semisocial: miembros de la misma generación viven juntos, cooperan en el cuido de la progenie y hay castas reproductivas; y por último 5- eusocialidad, considerada como la forma más compleja y derivada de comportamiento social (Rypstra 1993), caracterizada por generaciones de adultos que traslapan y cooperan en el cuido de crías. En esta clasificación existe la división reproductiva del trabajo y las castas, donde en ocasiones hay soldados no reproductivos y en otros casos sí se reproducen (Andersson 1984, Costa 2006, Rypstra 1993, Vollrath 1986).

Costa y Fitzgerald (1996) señalan que clasificar a las especies que viven en sociedad con base a la presencia o no de características eusociales, hacen que el resto de sociedades no-eusociales pierdan atractivo para los científicos, y en consecuencia, contribuye a generar

información sesgada e incompleta de la evolución social (Costa 2006). Se han escrito una gran cantidad de artículos sobre la eusocialidad y sus casos extremos en Hymenoptera (hormigas, abejas, avispas) e Isoptera (termitas), incluso sobre roedores como el caso de la rata topo desnuda (Andersson 1984, Costa 2006). El filo Arthropoda engloba un amplio espectro de comportamientos sociales complejos en los que, las etiquetas sub-, semi- y quasisocial difícilmente reflejan la complejidad de las interacciones de las sociedades en artrópodos (Costa 2006). Por lo tanto para entender la evolución de los sistemas sociales, es necesario explorar las condiciones que han sido seleccionadas, la relación costo-beneficio (Avilés y Salazar 1999), así como entender el origen de la socialidad, a partir de ancestros con rasgos sociales no tan extremos como los eusociales (Avilés *et al.* 2001).

Comportamiento social en arañas y su evolución. El comportamiento social en arañas está basado en grupos familiares que se originan a partir del cuido maternal extendido y de la dispersión tardía de los juveniles (Evans 1999, Bilde y Lubin 2001). Costa (2006) señala que en aproximadamente 50 familias de artrópodos y en más de una docena de órdenes, existen especies que cuidan de su progenie. Sin embargo, se considera que la socialidad en arañas es poco usual (Rypstra 1993). De las 47 000 especies descritas, menos de 30 muestran cooperación permanente (Avilés y Guevara 2017), ya que los adultos son principlmente solitarios y agresivos, incluso contra parientes (Vollrath 1986, Pasquet, Trabalon, Bagnères y Leborge 1997).

En arañas, los miembros de un grupo social cooperan en la construcción y mantenimiento de la tela, en la captura de presas y en el ya mencionado cuido maternal (Avilés *et al.* 2001). Entre los beneficios de vivir en grupo se encuentran, la protección contra depredadores, el mal clima y parásitos (Pasquet y Krafft 1992), compartir el costo del mantenimiento del nido (Pruitt y Avilés 2018), la posibilidad de capturar presas de mayor tamaño y explorar hábitats que no serían posibles para individuos solitarios (Avilés *et al.* 2001).

Para realizar la transición de un estilo de vida solitario a uno grupal, es necesario que las arañas de un mismo grupo no demuestren agresión en situaciones de competencia, y que los individuos mantengan la tolerancia entre sí (Bilde y Lubin 2001, Kullman 1972, Pasquet *et al.* 1997). Riechert (1985) señala que la socialidad en arañas pudo haber evolucionado en hábitats donde la selección a agresividad es menos fuerte en contextos como forrajeo. La socialidad en arañas ocurre casi exclusivamente en ambientes ricos en presas, y estos ambientes tienden a favorecer la evolución de fenotipos poco agresivos en especies solitarias de arañas (Riechert 1993) y en arañas coloniales (Uetz y Hieber 1997).

La socialidad en arañas ha evolucionado independientemente al menos 19 veces en 7 familias (Bilde y Lubin 2011) y se deriva a partir de formas subsociales con cuido maternal extendido y con algún tipo de cooperación entre los juveniles (Avilés 1997). Kullman (1972) lo denominó como la *ruta subsocial* de la evolución del comportamiento social; en donde, el cuido maternal extendido, la tolerancia a con-específicos y la habilidad restringida de dispersión, constituyen la base de la socialidad permanente (Agnarsson, Avilés, Coddington y Maddison 2006; Bilde y Lubin 2001, Bilde y Lubin 2011).

A diferencia de los insectos eusociales, las arañas sociales no poseen castas reproductivas (Agnarsson 2006). Avilés (1997) separó los comportamientos sociales de las arañas basada en dos aspectos: el periodo de coexistencia en una colonia (sociabilidad periódica o permanente) y si poseen o no territorios dentro de la colonia (territoriales o no territoriales). De acuerdo a lo anterior Avilés *et al.* (2001) clasifican a las arañas en cuatro categorías relativamente discretas:

1. Territoriales de sociabilidad periódica: agregaciones de telas individuales donde individuos se dispersan antes del apareamiento, como por ejemplo el aranéido *Parawixia bistriata* (D'Andrea 1987).

2. Territoriales de sociabilidad permanente: agregaciones de telas individuales que pueden mantenerse por varias generaciones, como *Cyrtophora* (tres especies) y Uloboridae (cuatro especies de *Philoponella*) (Uetz y Hieber 1997).
3. Subsociales o no territoriales de sociabilidad periódica: las crías de una hembra se mantienen juntas esté o no la madre en un nido común. Entre las familias con especies subsociales se encuentran Agelenidae, Theridiidae, Thomisidae, Pholcidae, entre otras (Avilés 1997).
4. Quasisociales o no territoriales de sociabilidad permanente: las crías y la hembra permanecen juntas en un nido común. Son las únicas arañas que muestran cuido parental como *Anelosimus eximius* (Avilés 1997) y para los efectos de este trabajo serán llamadas arañas sociales.

Las arañas sociales puede que no sean muy exitosas desde un punto evolutivo y ecológico (Agnarsson *et al.* 2006), sin embargo, algunos géneros muestran orígenes de socialidad en múltiples ocasiones. Experimentalmente pueden ser manipulados con facilidad y criados en grandes números en el laboratorio, lo que permite observar cambios en el crecimiento de las colonias, en su sobrevivencia, en las interacciones y otros aspectos biológicos, lo que no sería posibles con otros organismos (Pruitt y Avilés 2018).

Comunicación, señales químicas, selección y reconocimiento de parentela. La comunicación animal es el intercambio de información entre individuos de la misma o diferente especie, en donde el transmisor y receptor se benefician de alguna manera de tal intercambio (Greenfield 2002, Trabalon 2013). Wilson (1971) menciona que la comunicación de manera recíproca entre individuos con fines cooperativos es la esencia de la socialidad. En otras palabras, la comunicación es lo que mantiene a los individuos juntos y sin ella no puede haber una influencia positiva en el valor adaptativo de los demás, como ocurre en las agrupaciones de individuos, donde su valor adaptativo puede ser negativo o neutro (Costa 2006). En los grupos sociales existen mayores oportunidades para que ocurran interacciones repetidas entre individuos, por eso se espera que

ocurra una mayor ritualización, lo que ocasiona que la comunicación entre individuos sociales sea compleja (Lubin 1986).

La información es transmitida como señales o estímulos que pueden solicitar varios canales para ser recibidos (Trabalon 2013) sin embargo, es necesario diferenciar estímulo y señal antes de enfocarse en un tipo de canal. Un estímulo es todo aquello que emite un individuo, que incluye elementos visuales, vibraciones y ciertos químicos. A diferencia de una señal, los estímulos modifican el ambiente físico o químico inducido por el comportamiento especializado del transmisor e influyen en el comportamiento del receptor de tal manera que el transmisor se beneficia. No todos los estímulos son señales (Greenfield 2002).

A pesar del tamaño pequeño de los artrópodos, la comunicación química es sumamente eficiente y ventajosa sobre otros tipos de comunicación, debido a que los compuestos químicos se pueden transmitir por muchas formas, como por ejemplo a través de barreras y en una amplia variedad de rangos, así mismo, se puede regular su emisión y se pueden discriminar sus compuestos con un alto grado de sensibilidad (Greenfield 2002). Muchos estudios han demostrado que las arañas utilizan la comunicación química en varios escenarios como al buscar pareja, al iniciar el cortejo o durante la aceptación de pareja (Schulz 2013). Las arañas producen feromonas volátiles y de contacto. Las hembras producen señales volátiles para comunicarse con machos y otras hembras, mientras que las feromonas de contacto están típicamente asociadas a la tela o a sustratos (Trabalon 2013). La cantidad de feromonas identificadas en las arañas es muy baja, comparada con la cantidad identificada en los insectos (Schulz 2013). Barufaldi (2016) menciona que la primera feromona de araña, obtenida de *Linphia triangularis*, fue identificada casi 34 años después de las primeras feromonas identificadas en los insectos. Esta diferencia se puede atribuir al bajo impacto económico que ocasionan las arañas comparado al de insectos, así como a las dificultades experimentales durante el proceso de identificación, la baja cantidad de las feromonas producidas, entre otras (Schulz 2013).

Las arañas usan sus patas y palpos para oler y gustar, y es en los segmentos distales donde sus quimio receptores tienden a encontrarse (Schulz 2013). Los quimio receptores más importantes son los de contacto. Están constituidos por pelos quimio-sensitivos curvos, con puntas romas abiertas hacia el exterior. Cada uno posee usualmente 21 células sensitivas que se inervan, y son necesarios para el reconocimiento de presas, de pareja y para el reconocimiento entre mismos individuos (Trabalon 2013).

El reconocimiento de parentesco es una característica presente en el reino animal, mayormente en grupos sociales (Evans 1999). La habilidad para reconocer parentela implica que debe existir un estímulo confiable, el cual puede ser cualquier aspecto del fenotipo (Bilde y Lubin 2001), estos estímulos pueden ser heredados, ambientales o cualquiera que minimice errores de aceptación o rechazo (Sherman, Reeves y Pfennig 1997; Joseph, Snyder y Moore 1999). Entre los beneficios del reconocimiento de parentesco se encuentra la disminución de competencia entre parientes, así como el mantenimiento de la exogamia y la disminución del canibalismo (Johannesen y Lubin 1999). El reconocimiento de parentesco no significa que exista selección de parentela. Wade y Breden (1981) definen la selección de parentela como el proceso evolutivo que ocurre cuando individuos interactúan unos con otros por parentesco y esas interacciones afectan el valor adaptativo individual. Los individuos pueden aumentar su valor adaptativo mediante su reproducción (valor adaptativo directo) o indirectamente, por la reproducción de sus parientes (West, Griffin y Gardner 2007). Es más probable que ocurra selección de parentela cuando existe una mayor relación de parentesco dentro del grupo. Esto confiere mayores beneficios al valor adaptativo de los individuos que colaboran en la defensa del nido, en el forrajeo o en el cuido de la progenie (Lubin y Bilde 2007).

La cutícula de artrópodos posee compuestos como hidrocarburos, ácidos grasos, metil ésteres entre otros (Bagnères, Trabalon, Blomquist y Schulz 1997). Los individuos pertenecientes

a una misma colonia comparten un mismo olor colonial, el cual consiste de una mezcla específica de hidrocarburos de cutícula que puede variar entre colonias (Grinsted, Bilde y D'Ettorre 2011). La distinción entre los individuos se obtiene al comparar el perfil químico de un individuo, transferido a través del tacto, con el olor propio de la colonia (van Zweden y d'Etorre 2010). Basado en lo anterior Grinsted *et al.* (2011) sugieren que las arañas juveniles de *Stegodyphus lineatus* (araña subsocial de la familia Eresidae), reconocen a sus parientes por medio de los compuestos presentes en la cutícula. Así mismo, los perfiles cuticulares de *Anelosimus eximius*, una especie social, varían cuantitativamente entre colonias a pesar de no existir una aparente discriminación de parentela (Pasquet *et al.* 1997). Según Trabalon (2013), los sistemas y las señales que las arañas usan para el reconocimiento de parentesco no han sido investigados a profundidad. El estudio del reconocimiento de parentesco debe complementarse con estudios de comportamiento, como el realizado por Rypstra y Tirey (1989), en el cual encontraron que *Aneloimus domingo*, una especie subsocial, no tolera a los individuos de *Anelosmius eximius* cuando son introducidos artificialmente. Sin embargo, encontraron que ambas especies eran tolerantes hacia individuos de su misma especie provenientes de diferentes colonias. La habilidad para distinguir entre un miembro de un grupo o un intruso, es un elemento crítico del comportamiento social (Trabalon 2013). La comunicación tacto-química en arañas podría jugar un papel importante en la cohesión y organización de las arañas sociales, proporcionando pistas sobre la evolución de las señales químicas en las arañas (Pasquet *et al.* 1997).

Evolución de la tela en arañas. Prácticamente todas las arañas son depredadores de artrópodos (Blackledge *et al.* 2009) y en las arañas tejedoras, la tela es esencial para la captura de presas (Pasquet y Krafft 1992). Esta trampa inició como hilos delgados de seda sobre el suelo que una araña ancestral fue uniendo y pegando, pero no pudo hacer esto sin los patrones de comportamiento apropiados (Vollrath y Selden 2007).

Construcción y tipos de tela en arañas. Las telas de taxones más basales estaban restringidas al sustrato. Acontecimientos como el aumento en la regularidad geométrica, el comportamiento

estereotipado de hilar y la suspensión de las telas en el aire por medio de hilos estructurales permitió la evolución de las telas orbiculares (Blackledge *et al.* 2009). Por mucho tiempo se pensó que las telas orbiculares era lo más derivado en arañas. La aparición de la tela bidimensional, pegajosa orbicular, es considerada como una innovación clave para la diversificación de Orbiculariae (Agnarsson 2004). Esto ha llamado mucho la atención de científicos y ha generado que el resto de tipos de telas y su construcción no haya sido tan estudiada (Eberhard, Agnarsson & Levi 2008).

Entre las telas no orbiculares se encuentran las telas de plancha como la del agelénido *Melpomene* sp. (Eberhard y Hazzi 2017). Su comportamiento de construcción se basa en dos comportamientos: colocación de hilos de soporte y colocación de los hilos para llenar la plancha. Dentro de la familia Theridiidae existe gran variación en los tipos de tela (Eberhard, Agnarsson y Levi 2008). La tela ancestral de este grupo se cree fue similar a la de *Latrodectus* sp. Esta era una tela con pies de goma, un disco central del que salían líneas más o menos radiales, falta de una plancha y sin retiro en la periferia de la tela (Barrantes y Eberhard 2010). Otro ejemplo de construcción de tela irregular es el de *Tidarren sisyphoides* (Theridiidae). Madrigal-Brenes y Barrantes (2009) demostraron que el comportamiento de construcción de esta tela, constituye de cinco etapas que no son mutuamente excluyentes: exploración, suspensión del retiro, construcción del andamio, construcción de la plancha en forma de domo y construcción de la plancha baja horizontal.

Tela en arañas sociales. Se ha sugerido que la socialidad en arañas está asociada a las telas tridimensionales (Avilés 1997). El patrón geométrico de telas orbiculares no permite la participación simultánea de más de un individuo en su construcción, en cambio las telas tridimensionales pueden ser compartidas y los individuos pueden cooperar en su construcción, las crías de una o varias hembras pueden ser cuidadas, el movimiento entre sub telas es más sencillo, entre otras características que hace que la arquitectura de estas telas sea ideal para la evolución de la socialidad (Avilés *et al.* 2001; Bilde y Lubin 2011; Pruitt y Avilés 2018).

Biología de Anelosimus studiosus y Anelosimus baeza. Anelosimus (Simon 1891) pertenece a la familia Theridiidae, la cual es una de las familias más grandes de arañas con más de 2300 especies descritas distribuidas en 98 genéros (Platnick 2008), exhiben una gran diversidad morfológica, ecológica y de comportamiento (Agnarsson 2004). Eberhard *et al.* (2008) mostraron que algunos teridios poseen una gran diversidad intra-específica de sus telas, por ejemplo algunas con estructuras con pies de goma, planchas planas por debajo de hojas, con y sin hilos con goma, con o sin maraña, con o sin plancha, entre otras, lo que hace que sea un grupo ideal para el estudio de la evolución de la tela (Agnarsson 2004).

Anelosimus se caracteriza principalmente por la pérdida del colulus, crestas en el plato epiginal, un subconductor en el palpo epiginal, picos estridulatorios en el abdomen de los machos y no tienen hilos pegajosos en sus telas. Sus telas tienen forma de canasta con una plancha más o menos en forma de canasta que está cubierta por hojas vivas o muertas, y tienen una maraña sobre la plancha que intercepta insectos voladores (Agnarsson 2006). Este género es de gran interés porque contiene especies solitarias y especies con un amplio gradiente social (Marques, Vasconcelos-Netto y de Mello 1998). Los bosques neo tropicales parece han favorecido la evolución repetida de las socialidad, la cual ha sucedido al menos tres veces independientemente, y cuenta con al menos 8 especies sociales (quasi-sociales) (Agnarsson *et al.* 2006) de las 25 especies sociales conocidas en arañas (Bilde y Lubin, 2011).

Anelosimus studiosus pertenece al grupo *studiosus* que generalmente son más pequeñas que las arañas de otros grupos de *Anelosimus*. Su distribución va de USA a Argentina en una altitud de los 0 m a los 2500 m en una variedad de ambientes pero ausente de los bosques lluviosos de tierras bajas (Agnarsson 2006). Es una especie con socialidad facultativa con etapas solitaria y subsocial. Típicamente una única madre adulta está en el nido junto con su progenie (Brach 1977). Eventualmente toda la progenie se dispersa del nido, a menudo después de copular donde pueden

fundar un nuevo nido solitariamente o en grupo (Agnarsson 2006). *Anelosimus baeza* pertenece al grupo *jucundus* que generalmente es más grande que las del grupo studiosus y son predominantemente subsociales (Agnarsson 2006). Su distribución va de América del Norte a América del Sur entre los 200 m y 2500 m de altitud (Agnarsson 2006, Rao y Aceves-Aparicio, 2012) pero está ausente a menos de 600 m en el bosque lluvioso (Purcell y Avilés 2008). Sus telas se caracterizan por también tener forma de canasta y maraña en la parte superior junto con hojas secas incorporadas a la tela. Como no hay un sesgo sexual se asume que existe exogamia en esta especie (Agnarsson 2006).

Resultados y conclusiones destacadas

A continuación presento un resumen de cada uno de los artículos que componen esta tesis. Resalto los resultados y conclusiones más destacadas.

1. El potencial de los hidrocarburos cuticulares como estímulo para el reconocimiento de parentela en dos arañas subsociales del género *Anelosimus* (Araneae: Theridiidae). Un rasgo importante que permite a las sociedades animales compartir los beneficios de la cooperación es el reconocimiento entre los individuos del grupo y la discriminación de intrusos en grupos familiares. Los hidrocarbonos de cutícula permiten a los insectos eusociales por ejemplo, identificar a sus parientes y rechazar a quienes no lo son. Sin embargo, su función inicial fue la de proteger a los insectos contra la desecación. Estos compuestos presentan una gran oportunidad para estudiar la evolución de las señales, específicamente las químicas en arañas. Las arañas sociales evolucionaron de arañas subsociales y estudios previos a este han demostrado que las arañas potencialmente pueden estar utilizando los hidrocarburos de cutícula para reconocer a intrusos en sus nidos. En esta investigación demuestro que *Anelosimus* cf. *studiosus* y *Anelosimus baeza*, dos arañas con diferente nivel de socialidad, tiene perfiles químicos cuticulares complejos que son no solo específicos entre especies sino también particulares a cada colonia. Encontré también que

existen compuestos compartidos entre ambas especies y que *A.* cf. *studiosus* tiene menos compuestos pesados en su cutícula que *A. baeza*, lo que puede sugerir una conexión entre el nivel de tolerancia de la madre hacia su progenie y la cantidad de compuestos pesados en la cutícula. Por último, los alcanos lineares son una minoría en la cutícula de ambas especies, lo que podría sugerir que la mezcla de compuestos ramificados sea la que sirva como estímulo para el reconocimiento de parentesco y no un solo compuesto específico.

2. Patrones de comportamiento en la fijación de hilos de seda en la araña subsocial *Anelosimus baeza* (Araneae: Theridiidae). Un rasgo considerado ancestral en arañas es el de organizar en pequeños módulos el comportamiento. *Anelosimus baeza* es una araña subsocial que teje una tela tridimensional en forma de canasta. En el presente trabajo me concentré en los detalles de comportamiento de construcción de la misma y me fue posible describir patrones de fijación de hilos que no son posibles de determinar al solo mirar una tela completa. Encontré que algunos patrones de fijación de hilos de *A. baeza* tienen similitudes con arañas orbiculares y con otros teridios, probando así que el diseño de la tela no afecta los patrones de fijación. Por ejemplo, patrones como caminar por debajo de hilos y la identidad de las patas que sostienen hilos que serán conectados a la tela fueron observados tanto en arañas orbiculares y otros teridios como *Achaeranea tesselata*, *Tidarren sisyphoides* y *Latrodectus* sp., que cuentan con telas muy diferentes a la de *A. baeza*. Los teridios son conocidos por su gran flexibilidad en el comportamiento de construcción de tela y *A. baeza* mostró flexibilidad en los comportamientos de fijación así como destreza y movimientos estereotipados que son una necesidad para arañas orbiculares a la hora de construir sus telas. La combinación de ambos rasgos pudieron haber contribuido en el diseño de la tela tridimensional de *A. baeza*.

Literatura citada

Agnarsson, I. 2004. Morphological phylogeny of cobweb spiders and their relatives (Araneae, Araneoidea, Theridiidae). Zoological Journal of the Linnean Society 141: 447–626.

Agnarsson, I. 2006. A revision of the New World eximius lineage of *Anelosimus* (Araneae, Theridiidae) and a phylogenetic analysis using worldwide exemplars. Zoological Journal of the Linnean Society 146: 453–593.

Agnarsson, I., L. Avilés, J. A. Coddington & W. Maddison. 2006. Sociality in theridiid spiders: repeated origins of an evolutionary dead end. Evolution 60: 2342–2351.

Andersson, M. 1984. The evolution of eusociality. Annual Reviews of Ecology and Systematics 15:165–189.

Avilés, L. 1997. Causes and consequences of cooperation and permanent-sociality in spiders. Pp. 476–498. *In* Choe, J. & Crespi, B. (eds.). The Evolution of Social Behavior in Insects and Arachnids Cambridge University Press. Cambridge, USA.

Avilés, L. & J. Guevara. 2017. Sociality in Spiders. Pp.188–223. *In* D. Rubenstein & P. Abbot (eds.). Comparative Social Evolution. Cambridge University Press. New York, USA.

Avilés, L., W. Maddison, P. Salazar, G. Estévez, P. Tufiño, & G. Cañas. 2001. Arañas sociales de la Amazonía ecuatoriana, con notas sobre seis especies sociales no descritas previamente. Revista Chilena de Historia Natural 74: 619–638.

Avilés, L. & P. Salazar. 1999. Notes on the Social Structure, Life Cycle, and Behavior of *Anelosimus rupununi*. American Arachnological Society 27: 497–502.

Bagnères, A.G, M. Trabalon, G. Blomquist & S. Schulz. 1997. Waxes of the social spider *Anelosimus eximius* (Araneae, Theridiidae): abundance of novel n-propyl esters of long-chain methyl- branched fatty acids. Archives of Insect Biochemistry and Physiology 36: 295–314.

Barrantes, G. & W.G. Eberhard. 2010. Ontogeny repeats phylogeny in *Steatoda* and *Latrodectus* spiders. The Journal of Arachnology 38: 485–494.

Barufaldi, L. 2016. Function and diversity of sex pheromones in representative species of the black widow spiders *(*genus *Latrodectus, Araneae: Theridiidae).* (Unpublished doctoral dissertation) University of Toronto, Toronto, Canada.

Bilde, T. & Y. Lubin. 2001. Kin recognition and cannibalism in a subsocial spider. Journal of Evolutionary Biology 14: 959–966.

Bilde, T. & Y. Lubin. 2011. Group living in spiders: cooperative breeding and coloniality. Pp. 275–306. *In* M. Herberstein (ed.). Spider Behaviour Flexibility and Versatility. Cambridge University Press. New York, USA.

Blackledge, T., N. Scharff, J. A. Coddington, T. Szüts, J. W. Wenzel, C. Y. Hayashi, C. & I. Agnarsson. 2009. Reconstructing web evolution and spider diversification in the molecular era. Proceedings of the National Academy of Science 106: 5229–5234.

Brach, V. 1977. *Anelosimus studiosus* (Araneae: Theridiidae) and the evolution of quasisociality in theridiid Spiders. Evolution 31:154–161.

Costa, J. 2006. The other insect societies. Harvard University Press. Cambridge, USA.

Costa, J. & T. Fitzgerald. 1996. Developments in social terminology: semantic battles in a conceptual war. Trends in Ecology & Evolution 11: 285–289.

D'Andrea, M. 1987. Social behavior in spiders (Arachnidae, Araneae). Italian Journal of Zoology 3: 1-156.

Eberhard, W. G., I. Agnarsson & H. Levi. 2008. Web forms and the phylogeny of theridiid spiders (Araneae: Theridiidae): chaos from order. Systematics and Biodiversity 6: 415–475.

Eberhard, W. G. & N. A. Hazzi. 2017. Web building and prey wrapping behavior of *Aglaoctenus castaneus* (Araneae: Lycosidae: Sosippinae). Journal of Arachnology 45: 177–197.

Evans, T. 1999. Kin recognition in a social spider. Proceedings of the Royal Society of London 266: 287–292.

Greenfield, M. D. 2002. Signals and Receivers: Mechanisms and evolution of arthropod communication. Oxford University Press. New York, USA.

Grinsted, L., T. Bilde, & P. d'Ettorre. 2011. Cuticular hydrocarbons as potential kin recognition cues in a subsocial spider. Behavioral Ecology 22: 1187–1194.

Johannesen, J. & Y. Lubin. 1999. Group founding and breeding structure in the subsocial spider *Stegodyphus lineatus* (Eresidae). Heredity 82: 677–686.

Joseph, S. B., W. E. Snyder & A. J. Moore. 1999. Cannibalizing *Harmonia axyridis* (Coleoptera: Coccinellidae) larvae use endogenous cues to avoid eating relatives. Journal of Evolutionary Biology 12: 792–797.

Kullmann, E. J. 1972. Evolution of Social Behavior in Spiders (Araneae; Eresidae and Theridiidae). American Zoologist 12: 419–426.

Lubin, Y. 1986. Courtship and alternative mating tactics in a social spider. The journal of arachnology 14: 239–257.

Lubin, Y. & T. Bilde. 2007. The Evolution of Sociality in Spiders. Advances in the Study of Behavior 37: 83–146.

Madrigal-Brenes, R. & G. Barrantes. 2009. Construction and function of the web of *Tidarren sisyphoides* (Araneae: Theridiidae). Journal of Arachnology 37: 306–311.

Marques, E., J. Vasconcelos-Netto & M. de Mello. 1998. Life History and Social Behavior of *Anelosimus jabaquara* and *Anelosimus dubiosus* (Araneae,Theridiidae). American Arachnological Society 26: 227–237.

Pasquet, A. & B. Krafft. 1992. Cooperation and Prey Capture Efficiency in a Social Spider, *Anelosimus eximius* (Araneae, Theridiidae). Ethology 90: 121–133.

Pasquet, A., M. Trabalon, A. G. Bagnères, & R. Leborgne. 1997. Does group closure exist in the social spider *Anelosimus eximius*? Behavioural and chemical approach. Insectes Sociaux 44: 159–169.

Platnick, N. 2008. *The world spider catalogue*, version 9.0. American Museum of Natural History.

Pruitt, J. & L. Avilés. 2018. Social spiders: mildly successful social animals with much untapped research potential. Animal Behaviour 143: 155–165.

Purcell, J., & L. Avilés. 2008. Gradients of precipitation and ant abundance may contribute to the altitudinal range limit of subsocial spiders: Insights from a transplant experiment. Proceedings of the Royal Society B: Biological Sciences 275: 2617-2625.

Rao, D. & A. Aceves-Aparicio. 2012. Notes on the ecology and behavior of a subsocial spider *Anelosimus baeza* (Araneae: Theridiidae). American Arachnological Society 40: 325–331.

Riechert, S. 1985. Why do some spiders cooperate? *Agelena consociata*, a case study. Florida Entomologist 68 105–116.

Riechert, S. E. 1993. The evolution of behavioral phenotypes: Lessons learned from divergent spider populations. Advances in the Study of Behavior 22: 103–134.

Rypstra, A. 1993. Prey size, social competition, and the development of reproductive division of labor in social spider groups. The American Naturalist 142: 868–880.

Rypstra, A. & S. Tirey.1989. Observations on the social spider, *Anelosimus Domingo* (Araneae, Theridiidae), in southwestern Peru. Journal of Arachnology 17: 368–371.

Schulz, S. 2013. Spider Pheromones – A Structural Perspective. Journal of Chemical Ecology 39: 1–14.

Sherman, P. W., H. K. Reeves & D. W. Pfennig. 1997. Recognition systems. p. 69–96. *In* J. Krebs & N. Davis (eds.). Behavioral Ecology: An Evolutionary Approach. Blackwell Science Oxford, United Kingdom.

Trabalon, M. 2013. Chemical communication and contact cuticular compounds in spiders. Pp. 125–140. *In* W. Netwig (ed.). Spider Ecophysiology. Springer-Verlag. Berlin Heidelberg, Germany.

Uetz, G. & C. Hieber. 1997. Colonial web-building spiders: balancing the costs and benefits of group living. Pp. 458–475. *In* J. Choe & B. Crespi (eds.). The evolution of social behavior in insects and arachnids. Cambridge University Press. Cambridge, United Kingdom.

Van Zweden, J. & P. d'Etorre. 2010. Nestmate recognition in social insects and the role of hydrocarbons. p. 222–243. *In* G. J. Blomquist & A. G. Bagnères (eds.). Insect hydrocarbons: biology, biochemistry and chemical ecology. Cambridge University Press. Cambridge, United Kingdom.

Vollrath, F. & P. Selden. 2007. The Role of Behavior in the Evolution of Spiders, Silks, and Webs. Annual Review of Ecology, Evolution, and Systematics 38: 819–846.

Wade, M. & F. Breden. 1981. Effect of inbreeding on the evolution of altruistic behavior by kin. Evolution 35: 844–858.

West, S. A., A. S. Griffin & A. Gardner. 2007. Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection. Journal of Evolutionary Biology 20: 415–432.

Wilson, E.O. 1971. The Insect Societies. Harvard University Press. Cambridge, USA.

ARTICLE 1

Potential role of Cuticular Hydrocarbon Compounds in kin recognition of two subsocial *Anelosimus* spiders (Araneae: Theridiidae)

El potencial de los hidrocarburos cuticulares como estímulo para el reconocimiento de parentela en dos arañas subsociales del género *Anelosimus* (Araneae: Theridiidae)

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Abstract. Discrimination of non-group members is an important trait that allows animal societies to share the benefits of cooperation only between them. Cuticular hydrocarbon compounds allow eusocial insects to identify and reject non-kin and non-nestmates but their original function was to protect insects from desiccation. These compounds give an opportunity to study the role of chemical cues in social spiders. Social spiders are thought to have evolved from a sub-social state and previous studies have shown that spiders could potentially utilize compounds in their cuticles to recognize kin. Here I show that *Anelosimus* cf. *studiosus* and *Anelosimus baeza,* two subsocial spiders with different level of sociality, have complex cuticular chemical profiles that are species and colony-specific. I found that both species share cuticular compounds and that *Anelosimus* cf. *studiosus* have cuticles with less heavy compounds typical of spiders not ready to leave the nest. *A. baeza* showed heavier compounds, suggesting that extended periods of cooperation in more social species might be regulated by developmental changes in CHCs profiles. Lastly, single long chained alkanes are a minority in the cuticle of both spider species which suggests that quantitative and qualitative differences of branched alkanes might serve as kin recognition cues and not the long chained alkanes.

Keywords: branched alkanes, chemical cues, communication, tolerance

Arthropod cuticles are covered by waxy substances, most of this substances are long chained non-volatile hydrocarbons, named Cuticular Hydrocarbon Compounds (CHCs), that probably evolved originally to avoid desiccation (Blomquist & Bagneres 2010, d'Ettorre & Lenoir 2010, Trabalon 2013) and have later evolved into signals for chemical communication in arthropods (Greenfield 2002). A large number of studies in social insect species (Dani *et al.* 2005), have demonstrated that these compounds have a function in kin and nestmate recognition (Greenfield 2002; van Zweden, Dreier & d'Ettorre, 2009; Richard & Hunt 2013).

Spiders use chemical information to communicate in various scenarios (Schulz 2013). Female spiders for example use volatile chemicals aimed to communicate with males or other females, silk and body cuticles emit volatile information as well and have contact pheromones that are received by other spiders through chemosensitive hairs in their palps and legs (Trabalon 2013).

Sociality in spiders has different levels or gradients based on the time they spend in the maternal nest and whether or not they maintain individual territories in the colony (Avilés *et al.* 2001). The social behavior of spiders includes some of these characteristics: cooperation in feeding, prey capture, construction of the web and brood care, there are a few species that overlap generations but there is not reproductive division of labor (Avilés & Salazar 1999; Lubin & Bilde 2007) however Wright, Holebrook and Pruitt (2014) found evidence that task specialization might be linked with personality in a subsocial spider. Spiders are therefore candidates to use these compounds as signals to allow them to share the benefits of cooperation only with colony members (Howard & Blomquist 2005; d'Etorre & Lenoir 2010; Grinsted, Bilde & d'Etorre 2011). Pasquet *et al.* (1997) and Grinsted *et al*. (2011) found quantitative differences in the chemical profiles between family groups of the same species of social and subsocial spiders respectively. This suggests that chemical composition of the cuticles in social spiders and subsocial spiders, may contain information on family identity. For the effects of this paper, social spiders are those that are nonterritorial, do not disperse at maturity, stay in the communal nest and have parental care. Subsocial spiders are defined as nonterritorial spiders that stay in the communal nest but disperse, the moment depends on the species (Aviles *et al*. 2001).

According to Greenfield (2002), a stimuli or a cue is only designated as a signal when a stimuli undergoes evolutionary modifications and individuals derive enhanced benefits from emitting and receiving the stimuli. For example, a cuticle profile often ranges from highly complex to relatively simple (Dani *et al.* 2001; Chung & Carroll 2015). In *Formica cunicularia* 81 different CHCs were found but only 10 in *Manica rubida*, but is unknown if all compounds are utilized as chemical cues in these two-ant species (Dani *et al.* 2001). Discrimination presumably occurs when

an individual perceives the chemical profile of an encountered individual different to that of its own colony profile (Grinsted *et al.* 2011). In spiders, the evidence regarding the function of cuticular chemical cues is limited to inferences made from behavioral observations that suggests that such chemical products could play a role in kin discrimination in social and subsocial spiders (Trabalon, Pourié & Hartman 1998, Gaskett 2007).

Cuticular chemical cues have been linked with tolerance in social insects, and have been proposed to be involved in social spider evolution as well. Trabalon (2013) found that the transition from the gregarious phase to the solitary phase in *Tegenaria atrica (Agelenidae*) is linked to a change in the tolerance of the mother in relation to changes in the composition of the cuticular compounds of the young. Trabalon (2013) found that virgin females have characteristic compounds that appear before dispersal. The study of chemical profiles is therefore fundamental to understand the evolution of chemical cues and recognition mechanisms in spiders (Ward & Webster 2016).

In this paper, I describe the cuticular profile of two subsocial *Anelosimus* species that differ in their level of subsociality. I tested the hypothesis that CHCs can function in the discrimination of species and kin. There are possible benefits of recognizing kin when living in a group; it could have potential benefits in cooperation (Lubin & Bilde 2007), aggressiveness towards intruders (van Zweden, Dreier & d'Etorre 2009) and the avoidance of inbreeding (Agnarsson, Avilés, Coddington & Maddison 2006). Under this hypothesis I expected that the CHCs be species specific and colony (family group) specific. This predicts as well that the magnitude of the variation in CHCs colony profile will correspond to the level of sociality of the species. I also tested that CHCs may regulate the level of tolerance mother to sibling and sibling to sibling. This predicts that in the more social species heavier compounds will be found as they might disperse later, mother or adults are more tolerant to the progeny staying longer.

MATERIALS AND METHODS

Study organisms. The majority of social spider species are in the family Theridiidae (Lubin & Bilde 2007), mainly in the genus *Anelosimus* which includes 53 described species, mostly in tropical or subtropical regions (Agnarsson, Maddison & Avilés 2007). Their social behavior has different levels and ranges from nonterritorial permanent social to solitary (Agnarsson 2006). It is generally accepted that sociality in spiders is derived evolutionarily from a subsocial state (Avilés 1997) and in *Anelosimus* there are multiple independent origins of sociality (Agnarsson, Avilés, Codington & Maddison 2006, Lubin & Bilde 2007). I chose for my experiments two *Anelosimus* species, *A.* cf. *studiosus* and *A. baeza*, with different level of subsocial behavior.

A. cf. *studiosus* is widespread and common from 0 to 2500 m, from USA to Argentina, absent in lowland tropical rainforests (Brach 1977, Agnarsson 2006), whereas *A. baeza* occur from 200 to 2500 m from Mexico to southern Brazil but absent below 600 m in tropical rainforests (Rao & Aceves-Aparicio 2012). Both species construct a basket type web at the end of branches on herbaceous vegetation and low branches of bushes and trees (Agnarsson 2006). *A.* cf. *studiosus* colonies are constituted mainly by a single mother and offspring in each nest and contain up to 50 spiders, although Pruitt, Riechert and Jones (2008) mention a variable social structure along a latitudinal gradient, apparently this spider shows a social behavior polimorfism. *A. baeza* also seems to have variable social behavior, but has not been studied as much as *A.* cf. *studiosus,* but sometimes *A. baeza* forms larger colonies but smaller compared to the social *A. eximius (*Agnarsson 2006). *A. baeza* colonies can have several adult females but sex ratio is not biased, unusual for colonies containing more than one female. The lack of sex ratios bias indicates outbreeding, males, females or both must exit their natal colony to seek mates (Agnarsson 2006). Furey (1998) and Viera, Ghione and Costa (2007) found female-biased sex ratio in multi female

nests of *A.* cf. *studiosus* who also seems to be less tolerant than *A. baeza*, as tend to pursue offspring out of the web the and do not tolerate adult females when they are not in multi female nests (Agnarsson 2006). In this study I worked with colonies of *A.* cf. *studiosus* that had only one adult female or it had disappeard, which is usual when juviles reach the 5^{th} instar (Agnarsson 2006) and for the purpose of this study are then categorized as less social than *A. baeza* that always contained more than one adult female in their colonies.

Spider Collection. In January 2014, eight *A.* cf. *studiosus* colonies for a total of 132 individuals and three colonies of *A. baeza* for a total of 58 individuals were collected in Costa Rica in Monteverde, Puntarenas from 1491 masl to 1667 masl in an altitudinal gradient (10°18′53.16″ N, 84°48′28.38″W). All colony sites were marked with a GPS (Garmin etrex 10) and the entire colonies were collected and placed into plastic bags. Then taken directly from the field into a freezer at -10^0C to preserve them for cuticular chemical analyses.

Chemical analyses. To describe the cuticular chemical profiles of both species and then to compare them between colonies of the same species, I quantified variation in CHCs profiles of adults and juveniles of both sexes. For the analyses, I used between 18 and 20 spiders from each colony.

Each dead spider was immersed individually in clean screw cap vials (CZT) containing 1mL of pentane (n-Pentane for organic trace analysis UniSolv) for 1 minute. The animal was removed, and the solvent was stored at -40^0C until chemical analysis. Then the solvent was allowed to evaporate under a fume hood at room temperature to 100uL in case of adult females and adult males; for immature females samples were allowed to evaporate to 30uL using gas Nitrogen also under the fume hood. The content of each vial was transferred to crimp-top vials.

I added C-18 as a standard to all the samples in the following proportions: 0.1 uL C-18 +0.9 uL Hexane. For analysis, 1 uL of this mixture was injected into an Agilent Technologies 7890A gas chromatograph (GC, capillary column: HP-5MS Ultra Inert 30 m x 250 um x 0.25 um; splitless injector; carrying gas: helium at 1ml/min), with an Agilent Technologies 7683B Series

injector. The initial temperature was 50^0C and was increased at a rate of 10^0C/min to 200^0C, then to 310^0C at 4^0C/min. The GC was coupled to a Mass Spectrometer (MS, 70 eV electron impact ionization). Chemical compounds were identified on the basis of their retention time (compared with standards) and by inspecting diagnostic ions in their mass spectra.

Statistical analysis. In order to analyze the chemical profile of the spiders, 54 regularly occurring gas chromatography-mass spectrometry peaks for *Anelosimus* cf. *studiosus* and 44 regularly occurring gas chromatography-mass spectrometry peaks for *Anelosimus baeza* were integrated using Agilent Technologies Chem Station software. The normalized peak areas within each profile were calculated according to Grinsted *et al.* (2011) using the formula:

$$Z_{ij} = ln\left[\frac{Y_{ij}}{g(Y_j)}\right]$$

In brief Z_{ij} is the transformed area of peak *i* for individual *j*; Y_{ij} is the area of peak *i* for individual *j*; and $g(Y_j)$ is the geometric mean of the areas of all peaks for individual *j*.

These normalized peak areas were used as variables. I reduced the number of variables (compounds) using the loadings of Principal Component Analyses (PCA). A total of 27 regularly occurring peaks were cut from subsequent analysis based on their PC loadings (for the entire list of compound loadings see Table 1). The remaining compounds were analyzed using Discriminant Function Analyses (DFA) and MANOVA tests performed in PAST 3.12 (Hammer *et al*. 2001). To test the magnitude of the variation between chemical profiles I did Generalized Linear Models using the first and second PC with species and colonies as effects performed in STATISTICA 7.1 (StatSoft Inc.) ([mod1<-glm(PC1~species), mod1<-glm(PC2~species), mod1<-glm(PC1~colony) y mod1<-glm(PC2~colony]).

Chemical compounds that vary more between groups of individuals (colonies or species) than within groups are likely informative for group identity and hence may represent potential recognition cues (Grinsted *et al.* 2011). To identify the best candidates for kin recognition cues, the diagnostic power (DP) of each GC peak was calculated following van Zweden *et al.* (2009)

where in brief the standard deviation of the standardized peak area over all colonies was divided by the pooled standard deviation within these colonies. Peaks with higher-than-average DP are considered as "high DP" compounds which are the most variable between colonies, but comparatively most consistent within colony, and are therefore the most likely to act as kin recognition cues (van Zweden *et al.* 2009). For the purposes of this study all compounds with a chain length equal or longer than n-C25 (Grinsted *et al.* 2011) are consider to be heavy compounds based on retention times.

RESULTS

The molecular chemical profile of *A.* cf. *studiosus* was characterized by 43 species-specific peaks while *A. baeza* consistently showed 33 species-specific occurring peaks. Eleven additional peaks were regularly found in both species (Table 2) for a total of 87 peaks that can be identified as hydrocarbons. The classes of hydrocarbons could be: linear alkanes and branched alkanes as fatty acids are usually not found in spider cuticles (Trabalon *et al.* 1996; Pourie, Ibarra, Francke & Trabalon 2005; Grinsted *et al.* 2011), all with chain lengths ranging between *n*-C_8 and *n*-C_{33} (Fig. 1, Fig. 2). Only four compounds were identified by its name, all of them were linear alkanes, the rest were given a Peak ID.

Between species variation of the chemical profile

Spiders collected showed species-specific CHCs profiles. Chemical variation among species was due to qualitative and quantitative differences between chemical profiles. The main function (PC1; explaining 57.73% of the total variance) clearly separated the two groups (Fig. 3). A DFA on selected compounds (Table 2) significantly differentiated the two species (Wilks' λ: 0.02859; df1, df2 (60, 129); Approx. F (73.05); $p<0.0001$) and 97.89% of the samples were correctly assigned to their species.

Between colony variation of the chemical profile

Colonies showed specific CHCs profiles (Fig. 4). DFA on selected compounds differentiated all colonies (Wilks' λ: 1.73E-9; df1, df2 (600, 1216); Approx. F (13.62); $p<0.0001$) and 87.89% of the spiders were correctly assigned to their own colony. The DP of the 87 compounds ranged from 2.06 to 0.99.

From the totality of *A.* cf. *studious* chemical profile, 48% are considered to be high DP compounds ($DP_{average}$= 1.25). Nine of those were shared compounds and eight of the species specific high DP compounds are considered heavy compounds and ranged from *n*-C_{25} to *n*-C_{33}, the highest DP for this species was labeled as 28 ($DP_{peak\ Y10}$=2.03) and was a shared compound with a max *n*-C_{27} chain length (Fig. 1). (A list of all compounds ranked according to their DP is available in Table 3).

The chemical profile of *A. baeza* showed that 55% of the totality of its compounds have high DP values ($DP_{average}$= 1.30). Seven of the eleven shared compounds are considered high DP compounds. Almost all of the species specific high DP compounds ranged from *n*-C_{25} to *n*-C_{30} except for compounds labeled 4 and 5. The peak with the highest DP was peak labeled 28 ($DP_{peak\ B19}$=2.06) which was not a shared compound but have a max *n*-C_{26} chain (Fig. 2). In summary, *A. baeza* shows heavier compounds in its cuticle than *A.* cf. *studious* (A list of all compounds ranked according to their DP is available in Table 4).

Magnitude of the variation between CHCs colony profile

A. baeza and *A.* cf. *studiosus* showed difference in the magnitude of the variation between CHCs species profiles (GLM PC1, species F=205.16; df=1; $p<0.0001$/ GLM PC2, species F=2.55 df=1; p=0.11) and CHCs colony profiles (GLM PC1, colonies F=67.05; df=10;

p<0.0001/ GLM PC2, colonies F=4.47; df=10; p<0.0001). The magnitude of the variation of CHCs colony profiles of less social species is less than those of more social species.

Compound variation in the chemical profile

Only two peaks had high factor loadings on PC1 (factor loading > 0.5) these were: compound Y10, a shared compound and B24 a species-specific for *A. baeza*, both branched alkanes. Compound Y10 had high DP in *A.* cf. *studious* but low DP in *A. baeza* but was the high relative abundance of this compound in one of the colonies of the lather what separated them the most, suggesting that this compound may contain information not only in species identity but in colony-family identity as well.

Nine peaks belonged in a second group of high factor loadings (factor loading > 0.05), two of them were shared compounds, five species specific to *A. baeza* and two species specific to *A.* cf. *studiosus*. The rest of the compounds (including peak with label 28 in *A. baeza*) had low factor loadings on PC1 (all factor loadings <0.05), suggesting that in *A.* cf. *studious* the differences in concentration of all compounds is what separates colonies rather than only a group of compounds like in *A. baeza.*

DISCUSSION

Both *Anelosimus* species have different CHCs profiles, consisting mainly of long-chain branched hydrocarbons, and the combination of those compounds differs between species and between colonies. Several factors including age, sex, nutritional status and fluctuations in abiotic conditions are known to cause qualitative and quantitative changes in CHCs profiles (Chiara, Portugal & Jeanson 2019). The differences in chemical profiles between the colonies of both species suggest that CHCs profiles carry information about family identity and potentially could

be used as cues or signals in kin recognition. To be labeled as signals, CHC's must meet two conditions proposed by Greenfield (2002): (1) individuals derive expected benefits from emitting and receiving that stimuli; (2) the stimuli have undergone evolutionary modifications that enhance the benefits derived from providing information to receivers and influencing their behavior. As has been reported in other studies, both species have vagrant males which might be able to find colonies by detecting odors and locate receptive females by contact with CHCs. Females could also benefit from these emissions as they could reject sibling males trying to mate and avoid inbreeding, a phenomenon that occurs in social spider species, and has been pointed out as the reason for an evolutionary dead end in several social lineages (Agnarsson *et al.* 2006). I do not have evidence to support this hypothesis but my data suggest that CHCs profiles might be important in kin recognition in both sub-social spiders.

Colony-specific odors could be potentially used in recognition of siblings. This could have implications for social living and cooperation of both studied species. In spiders, social living and cooperation are associated with benefits such as catching larger prey, better protection from predators, cooperation on the construction of the communal web and raise communally of the young (Lubin & Bilde 2007). Evans (1999) mentions that kin recognition does not exist in social spiders, because non-kin are unlikely to be found in the group. This could be true for some species with poor dispersal like *A. eximius* and *Agelena consociate* (Avilés 1997) and has even been proved in the social phenotype of *A. studiosus* by Riechert and Jones (2008). The acceptance of immigrants into subsocial spider groups poses the question of why social species might be sacrificing the potential benefits of kin recognition, especially the ones on avoiding disease and optimal outbreeding (Evans 1999) when their subsocial states seem to be benefiting from it.

The magnitude of variation between colonies suggest a potential link between CHCs and tolerance in these two species. Depending on the species, sub-social spiders differ on the age of dispersal. A hypothesis regarding CHCs states that these compounds regulate the level of tolerance mother to sibling and sibling to sibling (Trabalon *et al.* 1996; Pourie & Trabalon 2001). Adult

spiders of *A.* cf. *studiosus* are less tolerant to their progeny than those of *A. baeza* (Agnarsson 2006). Grinsted *et al.* (2011) also found that in *S. lineatus* the chemical profile of the cuticle changes during the first 50 days of development. I found that more species specific high DP compounds were heavy compounds in *A. baeza*, heavier compounds are expected in *A.* cf. *studiosus* but only before dispersal. The relative proportions of longer alkanes increase with age, longer hydrocarbons could occur mainly to prepare the cuticle for the risky dispersal stage (Gibbs, Mousseau & Crowe 1991, Van Zweden *et al.* 2010, Trabalon 2013). This change in the cuticle might be the cue that could make adult females in *A.* cf. *studiosus* less tolerant but tests to determine how much age affects the chemical profile, especially in those species that have facultative sociality like *A.* cf. *studiosus* are needed. In my investigation *A. baeza* shows heavier compounds, what could be happening is that developmental changes in cuticular compound composition could be important in extending the cooperative stage of tolerance towards siblings in this subsocial spider (Trabalon 2013). Tests to determine those changes in cuticular composition are important to test this hypothesis.

Linear alkanes have repeatedly been found to play a less relevant role in kin recognition in several social insects (van Zweden *et al.* 2009), as well as in a subsocial spider (Grinstead *et al.* 2011). My results indicate that only a minority of the compounds of both species are linear alkanes. In *A.* cf. *studiosus* both linear alkanes found in the cuticle had low DP but in *A. baeza* *n*-C_{26} had high DP, this compound is also present in *S. lineatus* (Grinsted *et al.* 2011) but with low DP, the opposite occurs with *n*-C_{25}. This suggests that the distinctive CHCs profile of not only species but also groups of related individuals is the result of the combination of different compounds rather than the presence or absence of a single compound. *Anelosimus* species did not seem to share linear alkanes, and these ones as I mentioned, might be difficult to differentiate or simply not relevant to be recognized as cues. Selection might be happening in the receptors for branched compounds instead. Qualitative and quantitative differences of branched alkanes seems to be the signature of the species and or the family group. Signature smell has been demonstrated in mammals and birds and have been linked with physiological or environmental factors (Leclaire *et al.* 2011). Tests to

identify the specific CHCs signature of species and groups and how spiders react to this cue or cues must be made to further prove this hypothesis.

In conclusion, this study shows first that *A.* cf. *studiosus* and *A. baeza* have complex CHCs profiles that are species and colony specific. These specific profiles could have beneficial implications in the tolerance and consequential extension of group living and cooperation, as both species of spiders could be benefitting from kin-biased behavior and the maintenance of outbred population structure (Johannesen & Lubin 1999). Second, that developmental changes in CHC composition might extend the cooperative stage of tolerance in more social spiders like in *A. baeza* (Trabalon 2013). Tests following specific compounds through development, genetic relatedness experiments, bioassays testing if recognition cues perceived by spiders may be based on signature CHCs like Dani *et al.* (2005) found on other social insects and if CHCs profiles affect tolerance/aggression are needed to reinforce what my results suggest. That subsocial spiders could use CHCs as cue for kin-based behaviors that could favor the evolution of cooperation and group living. Thus, as Bilde & Lubin (2001) mention, the study of kin recognition in subsocial spider species is particularly interesting in the context of the evolution of sociality.

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LITERATURE CITED

Agnarsson, I. 2006. A revision of the New World eximius lineage of Anelosimus (Araneae, Theridiidae) and a phylogenetic analysis using worldwide exemplars. Zoological Journal of the Linnean Society 146: 453–593.

Agnarsson, I., L. Avilés, J. A. Coddington, & W. P. Maddison. 2006. Sociality in theridiid spiders: repeated origins of an evolutionary dead end. Evolution 60: 2342–2351.

Agnarsson, I., W. P. Maddison, & L. Avilés. 2007. The phylogeny of the social Anelosimus spiders (Araneae: Theridiidae) inferred from six molecular loci and morphology. Molecular Phylogenetics and Evolution 43: 833–851.

Avilés, L. 1997. Causes and consequences of cooperation and permanent-sociality in spiders. Pp. 476–498. *In* Choe, J. & B. Crespi (eds). The evolution of social behavior in insects and arachnids. Cambridge, UK.

Avilés, L. & P. Salazar. 1999. Notes on the Social Structure, Life Cycle, and Behavior of *Anelosimus rupununi*. American Arachnological Society 27: 497–502.

Avilés, L., W. Maddison, P. Salazar, G. Estévez, P. Tufiño, & G. Cañas. 2001. Arañas sociales de la Amazonía ecuatoriana, con notas sobre seis especies sociales no descritas previamente. Revista Chilena de Historia Natural 74: 619–638.

Bilde, T. & Y. Lubin. 2001. Kin recognition and cannibalism in a subsocial spider. Journal of Evolutionary Biology 14: 959–966.

Blomquist, G. & A. Bagneres. 2010. Insect Hydrocarbons Biology, Biochemistry, and Chemical Ecology. Cambridge University Press. Cambridge, UK.

Brach, V. 1977. Anelosimus studiosus (Araneae : Theridiidae) and the Evolution of Quasisociality in Theridiid Spiders. Evolution 31: 154–161.

Chiara, V., F. R. Portugal & R. Jeanson. 2019. Social intolerance is a consequence, not a cause of dispersal in spiders. PLoS Biol 17: 1–27.

Chung, H. & S. B. Carroll. 2015. Wax, sex and the origin of species: Dual roles of insect cuticular hydrocarbons in adaptation and mating. BioEssays 37: 822–830.

d'Ettorre, P. & A. Lenoir. 2010. Nestmate Recognition. p. 194–209. *In* L. Lach, C.L. Parr & K.L. Abbott. (eds) Ant Ecology. Oxford University Press. New York, USA.

Dani, F. R., G. R Jones, S. Corsi, R. Beard, D. Pradella, & S. Turillazzi. 2005. Nestmate Recognition Cues in the Honey Bee : Differential Importance of Cuticular Alkanes and Alkenes. Chem Senses 477–489.

Dani, F. R., G. R. Jones, S. Destri, S. H. Spencer & S. Turillazzi. 2001. Deciphering the recognition signature within the cuticular chemical profile of paper wasps. Animal Behaviour 62: 165–171.

Evans, 1999. T. 1999. Kin recognition in a social spider. Proceedings of the Royal Society of London 266: 287–292.

Furey, R. E. 1998. Two cooperative social populations of the theridiid spider Anelosimus studiosus in a temperate region. Animal Behaviour 55: 727–735.

Gaskett, A. C. 2007. Spider sex pheromones: emission, reception, structures, and functions. Biological Reviews of the Cambridge Philosophical Society 82: 27–48.

Gibbs A., T.A. Mousseau & J.H. Crowe. 1991. Genetic and acclimatory variation in biophysical properties of insect cuticle lipids. Proceedings of the National Academy of Sciences 88: 7257–7260.

Greenfield, M. D. 2002. Signals and Recivers: Mechanisms and evolution of arthropod communication. Oxford University Press. New York, USA.

Grinsted, L., T. Bilde & P. d'Ettorre. 2011. Cuticular hydrocarbons as potential kin recognition cues in a subsocial spider. Behavioral Ecology 22: 1187–1194.

Hammer, Ø., D. A. T. Harper, & P. D. Ryan. 2001. Paleontological statistics software package for

education and data analysis. Palaeontologia Electronica 41: 9–18.

Howard, R. W. & G. J. Blomquist. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annual Review of Entomology 50: 371–93.

Johannesen, J. & Y. Lubin. 2001. Evidence for Kin-Structured Group Founding and Limited Juvenile Dispersal in the Sub-Social. American Arachnological Society 29: 413–422.

Leclaire, S., T. Merkling, C. Raynaud, G. Giacinti, J. M. Bessière, S. A. Hatch & E. Danchin. 2011. An individual and sex odor signature in kittiwakes? Study of the semiochemical composition of preen secretion and preen down feathers. Naturwissenschaften 98: 615–624.

Lubin, Y. & T. Bilde. 2007. The Evolution of Sociality in Spiders. Advances in the Study of Behavior 37: 83–146.

Pasquet, A., M. Trabalon, A. G. Bagnères, & R. Leborgne. 1997. Does group closure exist in the social spider Anelosimus eximius? Behavioural and chemical approach. Insectes Sociaux 44: 159–169.

Pourie, G., F. Ibarra, W. Francke & M. Trabalon. 2005. Fatty acids mediate aggressive behavior in the spider *Tegenaria atrica.* Chemoecology 15: 161–166.

Pourie, G., & M. Trabalon. 2001. Plasticity of agonistic behaviour in relation to diet and contact signals in experimentally group-living of *Tegenaria atrica*. Chemoecology 11: 175–181.

Pruit, J. N., S. E. Riechert & T. C. Jones. 2008. Behavioural syndromes and their fitness consequneces in a socially polymorphic spider, *Anelosimus studiosus*. Animal Behaviour 76: 871–879.

Rao, D., & A. Aceves-Aparicio. 2012. Notes on the ecology and behavior of a subsocial spider Anelosimus baeza (Araneae : Theridiidae) in Mexico. American Arachnological Society 40: 325–331.

Richard, F. J. & J. H. Hunt, 2013. Intracolony chemical communication in social insects. Insectes Sociaux 60: 275–291.

Schulz, S. 2013. Spider Pheromones – A Structural Perspective. Journal of Chemical Ecology 39: 1–14.

Trabalon, M. 2013. Chemical communication and contact cuticular compounds in spiders. Pp. 125–140. *In* Netwig, W. (eds). Spider Ecophysiology. Springer-Verlag Berlin Heidelberg.

Trabalon, M., A. G. Bagnères, N. Hartmann & A. M. Vallet. 1996. Changes in cuticular compounds composition during the gregarious period and after dispersal of the young in *Tegenaria atrica* (Araneae, Agelenidae). Insect Biochemistry and Molecular Biology 26: 77–84.

Trabalon, M., G. Pourié, & N. Hartmann. 1998. Relationships among cannibalism, contact signals, ovarian development and ecdysteroid levels in *Tegenaria attica* (Araneae, Agelenidae). Insect Biochemistry and Molecular Biology 28: 751–758.

van Zweden, J. S., J. B. Brask, J. H. Christensen, J. J. Boomsma, T. A. Linksvayer, & P. d'Ettorre. 2010. Blending of heritable recognition cues among ant nestmates creates distinct colony gestalt odours but prevents within-colony nepotism. Journal of Evolutionary Biology 23: 1498–1508.

van Zweden, J. S., S. Dreier & P. d'Ettorre. 2009. Disentangling environmental and heritable nestmate recognition cues in a carpenter ant. Journal of Insect Physiology 55: 158–163.

Viera, C., S. Ghione & F. G. Costa. 2007. Mechanisms underlying egg-sac opening in the subsocial spider Anelosimus cf. studiosus (Araneae: Theridiidae). Ethology Ecology & Evolution 19: 61–67.

Ward, A., & M. Webster. (2016). Sociality: The Behaviour of Group Living Animals. Springer International Publishing. Switzerland.

Wright, C. M., C. T. Holebrook & J. N. Pruitt 2014. Animal personality alligns task specialization and task proficency in a spider society. Proceedings of the National Academy of Sciences. 111: 9533–9537.

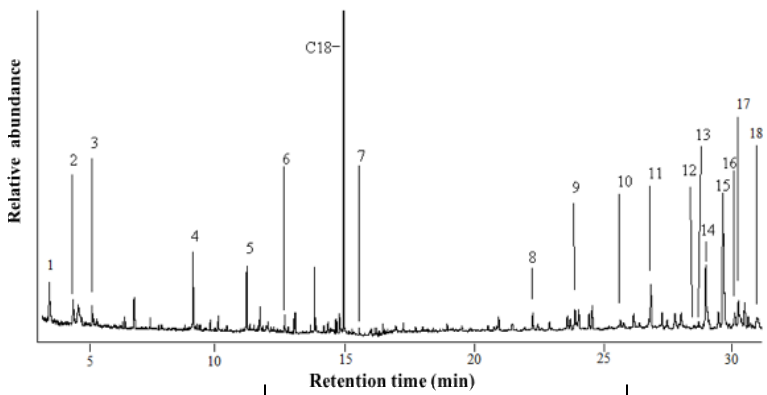
Appendix

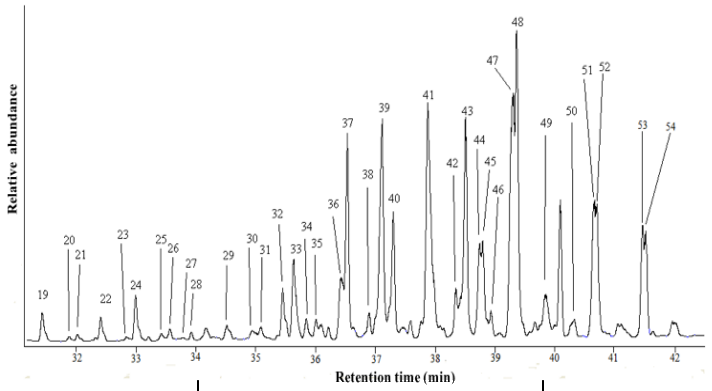
Table 1. Loadings obtained by the first PC for each compound of both spider species. Peak ID is the name or code given to the compound (Y=shared compound, A=*Anelosimus* cf. *studiosus*, B=*Anelosimus baeza*) whereas the grey mark means compounds < $|0.005|$ that were eliminated from the statistical analysis. Compounds with ** mean they belong in a high factor loading group (factor loading > 0.50) and * means they belong in a second group of high factor loadings (factor loading > 0.05).

Peak ID	Loading	Peak ID	Loading	Peak ID	Loading	Peak ID	Loading	Peak ID	Loading
Y0	0.017	A4	-0.002	A12	-0.001	B25	0.007	A36	-0.003
Y1	0.009	B7	0.034	B19	0.018	A22	-0.036	B31	0.020
Y2	0.007	A5	-0.005	B20	0.026	B26	0.034	A37	-0.021
B	0.018	B8	0.001	Y7*	0.143	A23	-0.004	A38	-0.005
A	-0.006	B9	0.004	Y8*	0.100	B27	0.000	A39	-0.005
B0	0.012	A6	-0.001	A13	-0.002	B28	0.005	A40	-0.005
A0	-0.006	B10	0.013	B21	0.005	B29	0.001	A41	-0.004
Y3	0.003	B11	0.038	B22*	0.053	A24	-0.035		
Y4	0.000	A7	-0.001	Y9*	0.051	B30*	0.073		
Y5	0.001	B12	0.010	A14	-0.002	A25*	-0.075		
A1	-0.001	A8	-0.015	A15	-0.006	A26	-0.030		
B1	0.002	B13	0.013	Y10**	0.752	A27*	-0.130		
B2	0.001	B14	0.024	A16	-0.008	A28	-0.014		
A2	-0.001	B15	0.018	B23*	0.120	A29	-0.006		
B3	0.005	A9	-0.004	A17	-0.001	A30	-0.010		
Y6	0.002	B16*	0.061	A18	-0.005	A31	-0.002		
B4*	0.064	A10	-0.006	B24**	0.572	A32	-0.006		
B5	0.003	B17*	0.079	A19	-0.013	A33	-0.001		
B6	0.004	B18	0.032	A20	-0.014	A34	-0.008		
A3	-0.001	A11	0.000	A21	-0.007	A35	-0.014		

Table 2. Number of spiders and percentages for all eleven shared compounds as not all individuals showcased these compounds on their cuticular profile.

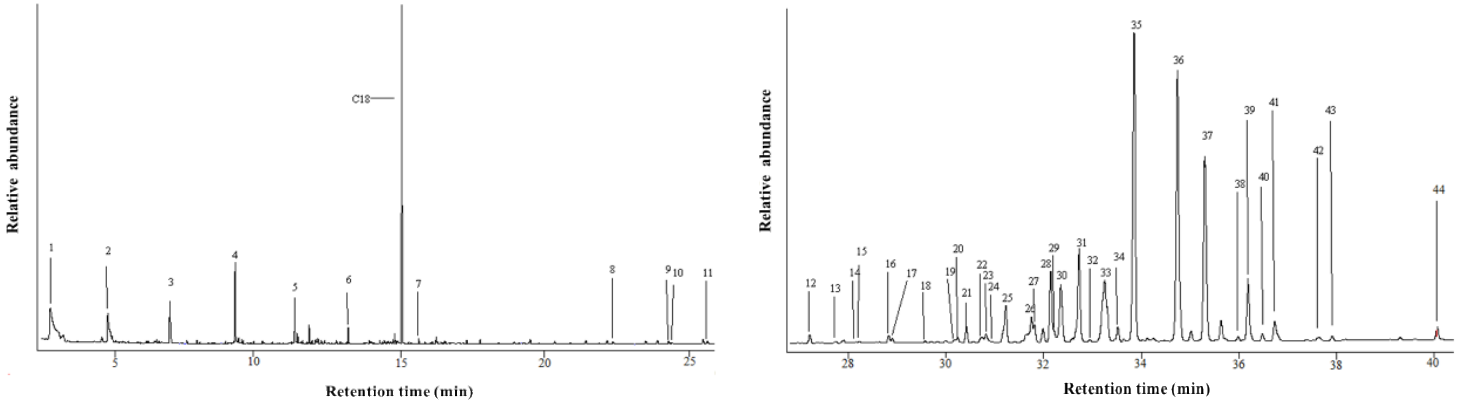
	Anelosimus cf. *studiosus*		*Anelosimus baeza*	
Peak ID	**Number of spiders with compound**	**Percentage (%)**	**Number of spiders with compound**	**Percentage (%)**
Y0	54	40.9	24	41.4
Y1	126	95.5	53	91.4
Y2	117	88.6	53	91.4
Y3	46	34.8	51	87.9
Y4	30	22.7	18	31
Y5	34	25.8	30	51.7
Y6	39	29.5	28	48.3
Y7	23	17.4	57	98.3
Y8	55	41.7	58	100
Y9	66	50	51	87.9
Y10	119	90.2	58	100





Label	Peak ID	DP	Label	Peak ID	DP	Label	Peak ID	DP	Label	Peak ID	DP	Label	Peak ID	DP
	n-C8			n-C18		17	A8	1.92	31	A18	1.09	46	A33	1.0
1	Y0	1.59		n-C19			n-C26		32	A19	1.52	47	A34	1.0
2	Y1	1.39		n-C20		18	A9	1.27	33	A20	1.26	48	n-C31	1.0
	n-C9		8	Y5	1.60	19	A10	1.14	34	A21	1.53	49	A36	0.99
3	Y2	1.27		n-C21		20	A11	1.01		n-C29		50	A37	1.0
	n-C10			n-C22		21	A12	1.35	35	A22	1.86	51	A38	0.99
	n-C11		9	A1	1.01	22	Y7	1.81	36	A23	1.01	52	A39	0.99
4	A	1.39		n-C23			n-C27		37	A24	1.29	53	n-C32	0.99
	n-C12		10	A2	1.52	23	Y8	1.75	38	A25	1.31	54	A41	0.99
5	A0	1.34	11*	Y6	1.07	24	A13	1.04	39	A26	1.08		n-C33	
	n-C13			n-C24		25	Y9	1.12	40	A27	1.57			
6	Y3	1.54	12	A3	1.06	26	A14	1.13		n-C30				
	n-C14		13	A4	1.60	27	A15	1.76	41	A28	1.0			
	n-C15			n-C25		28	Y10	2.03**	42	A29	1.03			
7	Y4	1.84	14	A5	1.89		n-C28		43	A30	1.0			
	n-C16		15	A6	1.00	29	A16	1.13	44	A31	1.0			
	n-C17		16	A7	1.50	30	A17	1.06	45	A32	0.99			

Fig. 1. A gas chromatograph broken in two, showing the cuticular hydrocarbon profile of an Anelosimus cf. studiosus spiderling. The panel below shows standards, marked in color grey. Label corresponds with their retention time Peak ID and the variation in chemical profiles between colonies expressed as DP of each compound.



Label	Peak ID	DP	Label	Peak ID	DP	Label	Peak ID	DP	Label	Peak ID	DP
	n-C8			n-C18		18	B9	1.83	34	Y9	1.25
1	Y0	1.59		n-C19		19	B10	1.08	35	Y10	1.09
2	Y1	1.39		n-C20		20	B11	1.12		n-C28	
	n-C9		8	Y5	1.60	21	B12	1.36	36	B23	1.7
3	Y2	1.27		n-C21		22	B13	1.9	37	B24	1
	n-C10			n-C22		23	B14	1.73		n-C29	
	n-C11		9	B1	1.03	24	n-C26	1.59	38	B25	1.01
4	B	1.39	10	B2	1	25	B16	1.43	39	B26	1.06
	n-C12			n-C23		26	B17	1.55	40	B27	1.55
5	B0	1.34	11	B3	1.09	27	B18	1.81	41	B28	1.65
	n-C13		12	Y6	1.06	28	B19	2.06**	42	B29	1.48
6	Y3	1.54		n-C24		29	B20	1.17	43	B30	1.22
	n-C14		13	B4	0.99	30*	Y7	1.52		n-C30	
	n-C15		14	B5	1.12		n-C27			n-C31	
7	Y4	1.84	15	B6	1.1	31	Y8	2.03**	44	B31	1
	n-C16		16	n-C25	1.12	32	B21	1.58		n-C32	
	n-C17		17	B8	1.21	33	B22	1.89		n-C33	

Fig. 2. A gas chromatograph showing the cuticular hydrocarbon profile of an *Anelosimus baeza* spiderling. The panel below shows standards marked in color grey, label corresponds with their retention time, Peak ID and the variation in chemical profiles between colonies expressed as DP of each compound.

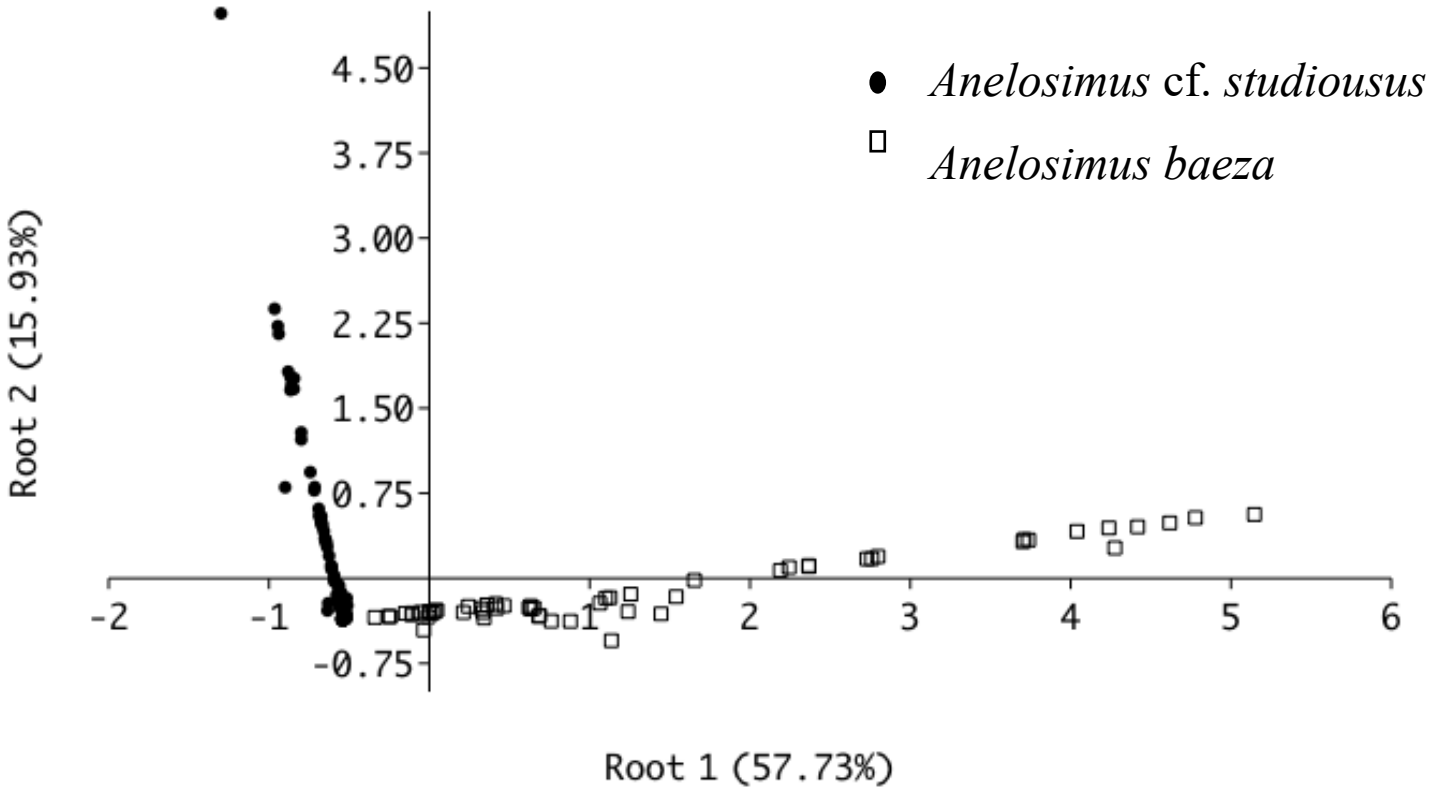


Fig. 3. Between species variation of the chemical profiles: a plot of the first 2 PC based on cuticular hydrocarbons from spiderlings and adults (190 individuals total). The percentage of the variance explained by each root is given in parenthesis.

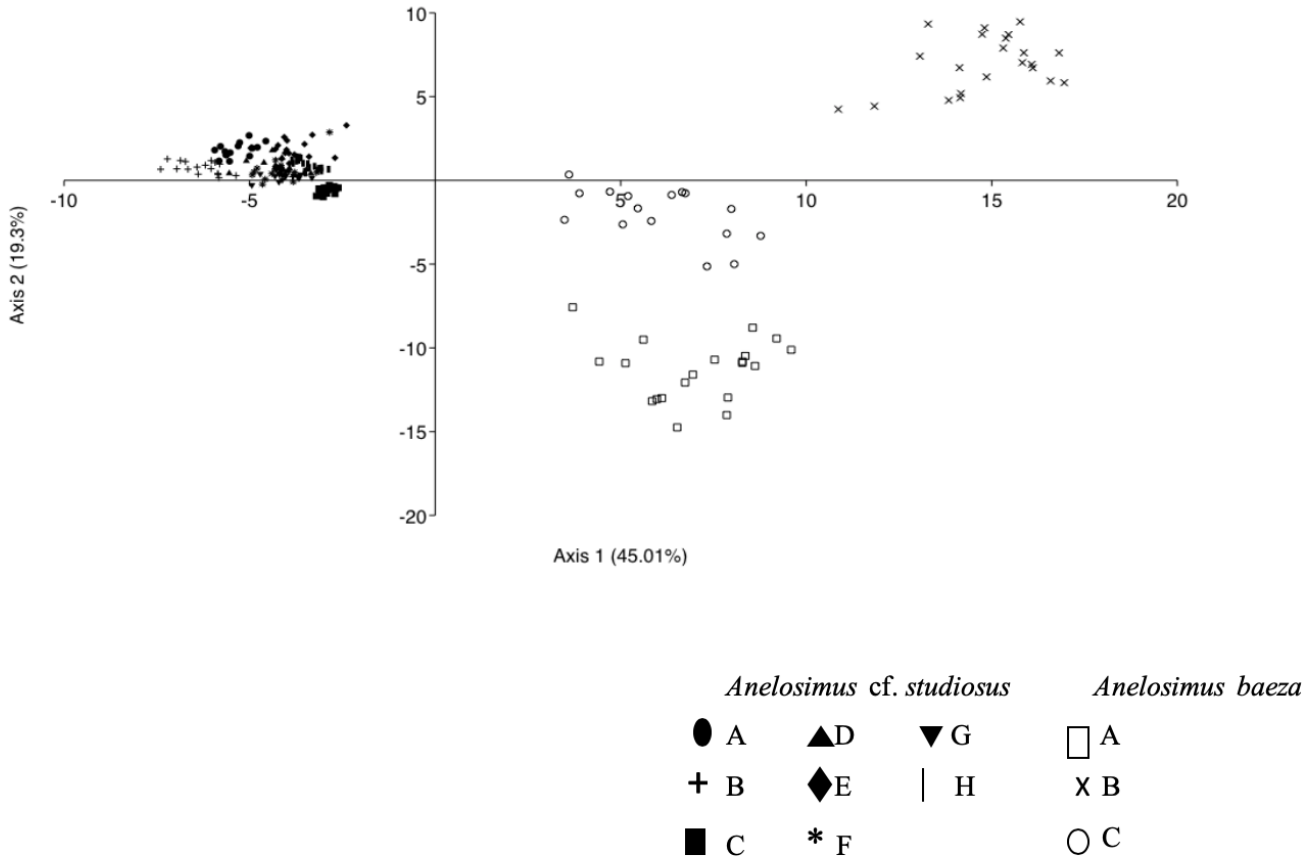


Fig. 4. Between colony variation of the chemical profiles: a plot of the first 2 axes of the DA based on cuticular hydrocarbons from spiderlings and adults (190 individuals total). Each symbol represents a colony. The percentage of the variance explained by each axis is given in parenthesis.

Table 3. Rank of compounds found in the cuticle of *Anelosimus* cf. *studiosus* according to their Diagnostic Power. Average DP was 1.25, compounds with the label equal or higher to 14 are considered heavy compounds based on retention times.

Rank	Label	Peak ID	DP	Ranking	Label	Peak ID	DP	Ranking	Label	Peak ID	DP
1	28	Y10	2.03	21	5	A0	1.34	41	15	A6	1
2	17	A8	1.92	22	38	A25	1.31	42	41	A28	1
3	14	A5	1.89	23	37	A24	1.29	43	43	A30	1
4	35	A22	1.86	24	3	Y2	1.27	44	44	A31	1
5	7	Y4	1.84	25	18	A9	1.27	45	46	A33	1
6	22	Y7	1.81	26	33	A20	1.26	46	47	A34	1
7	27	A15	1.76	27	19	A10	1.14	47	48	n-C31	1
8	23	Y8	1.75	28	26	A14	1.13	48	50	A37	1
9	8	Y5	1.6	29	29	A16	1.13	49	45	A32	0.99
10	13	A4	1.6	30	25	Y9	1.12	50	49	A36	0.99
11	1	Y0	1.59	31	31	A18	1.09	51	51	A38	0.99
12	40	A27	1.57	32	39	A26	1.08	52	52	A39	0.99
13	6	Y3	1.54	33	11	Y6	1.07	53	53	n-C32	0.99
14	34	A21	1.53	34	12	A3	1.06	54	54	A41	0.99
15	10	A2	1.52	35	30	A17	1.06				
16	32	A19	1.52	36	24	A13	1.04				
17	16	A7	1.5	37	42	A29	1.03				
18	2	Y1	1.39	38	9	A1	1.01				
19	4	A	1.39	39	20	A11	1.01				
20	21	A12	1.35	40	36	A23	1.01				

Table 4. Rank of compounds found in the cuticle of *Anelosimus baeza* according to their Diagnostic Power. Average DP was 1.30. Compounds with the label equal or higher to 16 are considered heavy compounds based on retention times.

Rank	Label	Peak ID	DP	Ranking	Label	Peak ID	DP	Ranking	Label	Peak ID	DP
1	28	B19	2.06	21	2	Y1	1.39	41	10	B2	1
2	31	Y8	2.03	22	4	B	1.39	42	37	B24	1
3	22	B13	1.9	23	21	B12	1.36	43	44	B31	1
4	33	B22	1.89	24	5	B0	1.34	44	13	B4	0.99
5	7	Y4	1.84	25	3	Y2	1.27				
6	18	B9	1.83	26	34	Y9	1.25				
7	27	B18	1.81	27	43	B30	1.22				
8	23	B14	1.73	28	17	B8	1.21				
9	36	B23	1.7	29	29	B20	1.17				
10	41	B28	1.65	30	14	B5	1.12				
11	8	Y5	1.6	31	16	n-C25	1.12				
12	1	Y0	1.59	32	20	B11	1.12				
13	24	n-C26	1.59	33	15	B6	1.1				
14	32	B21	1.58	34	11	B3	1.09				
15	26	B17	1.55	35	35	Y10	1.09				
16	40	B27	1.55	36	19	B10	1.08				
17	6	Y3	1.54	37	12	Y6	1.06				
18	30	Y7	1.52	38	39	B26	1.06				
19	42	B29	1.48	39	9	B1	1.03				
20	25	B16	1.43	40	38	B25	1.01				

ARTICLE 2

Dragline line attachment behavior patterns of the subsocial spider *Anelosimus baeza* (Araneae: Theridiidae)

Patrones de comportamiento en la fijación de hilos de seda por la araña subsocial *Anelosimus baeza* (Araneae: Theridiidae)

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Abstract. Organizing behavior into modules is suggested to be an ancient trait in spiders. By focusing on construction behavior details of *Anelosimus baeza* (Araneae:Theridiidae), a three-dimensional basket web builder, I was able to describe dragline attachment behavior patterns that couldn't be determined by just looking at the finished web. Observed patterns show similarities with both orb-weavers and other theridiids, proving that similar behavior patterns can be used to produce different web designs. For example the same legs held attachment lines and the dragline in orb-weavers and theridiids with strikingly different web designs. Theridiids are known for their flexible web building behavior, and *A. baeza* shows flexibility in dragline attachment behaviors, as well as dexterity a necessity in orb-weavers.

Keywords: behavioral patterns, web construction, behavior flexibility, basket web.

Sometimes it is easier to solve a problem by decomposing it into smaller sub-units, so that each can be solved individually; this method is used in control robotics based on animal behavior (Ratanaswasd, Dodd, Kawamura & Noelle 2006). Modularity occurs in spider web construction as semi-independent combinations of behavior patterns that are used by many spiders to construct their webs (Eberhard 2018). This trait of organizing behavior into modules is probably ancient in spiders (Eberhard in press).

Modular patterns occur at different level of analysis (Eberhard 2018). Construction behavior has been divided (somewhat arbitrarily) into two levels of detail: the order in which lines are added to the web; and the movements of different legs and how the lines are manipulated while the spider lays lines (Eberhard in press). In this paper I will focus in some details of web construction, by integrating the analysis of thread manipulation and leg movements, which could reveal underlying patterns that are difficult to perceive by just looking at the finished web (Vollrath & Selden 2007, Eberhard, Agnarsson & Levi 2008).

Leg movements as silk threads are attached have been observed mostly on orb-weavers (see Eberhard in press) and in a lesser extent in theridiids (see Lamoral 1968, Benjamin & Zschokke 2003, Jörger & Eberhard 2006, Eberhard, Barrantes & Madrigal-Brenes 2008 Madrigal-Brenes & Barrantes 2009). The hypothesis that organizing behavior into modules in spiders is ancient is supported by the distribution of attachment patterns and leg movements among different taxa. For example holding the dragline with a leg IV while walking is present in different aranenoid species (Eberhard 1982): *Acharanea tesselata* (Theridiidae) (Jörger & Eberhard 2006) and *Linyphia hortensis* (Linyphidae) (Benjamin & Zchokke 2004) and others (Eberhard in press). Another example is walk under lines holding the dragline with a leg IV and using legs on both sides, a behavior present in orb-weavers (Eberhard 1982), *Tidarren sisyphoides* (Theridiidae) (Madrigal-Brenes & Barrantes 2009), *Modisimus guatuso* (Pholcidae) (Eberhard 1992).

Web designs range in Araneoidea from simple lines to three-dimension structures. For instance species in the family Theridiidae construct a variety of webs (Eberhard *et al.* 2008), from extremely simplified as in *Phoroncidia studo* (Eberhard 1981), a web consisting of a single sticky line (Eberhard 1981) to extremely complex, including three-dimensional with aerial sheets (Saffre, Mailleux & Deneubourg 1999, Benjamin & Zschokke 2002, 2003, Jörger & Eberhard 2006). The large diversity in theridiid webs is associated with their great flexibility in microhabitat use, their ability to adjust the web design to different physical spaces, prey types, and prey availability (Jörger & Eberhard 2006, Agnarsson & Coddington 2007). Knowledge of the behavior used to build three-dimensional webs of theridiids are built is generally fragmentary and limited to only a few genera (Madrigal-Brenes & Barrantes 2009). Some descriptions of their webs as ‘highly irregular’ may have been because authors focused on the web and not on the construction behavior, which has often resulted more stereotyped than expected (Benjamin & Zschokke 2003).

This study provides a detailed description of the attachment of silk threads (e.g major ampulate threads) in the subsocial spider *Anelosimus baeza*, a three dimensional web builder spider (Agnarsson 2006). Their "basket" webs consists of a strong cupped sheet below an irregular tangle (Agnarsson 2006, Rao & Aceves-Aparicio 2012). Some details of the behavior associated attaching one thread to another is widespread across araneoids including other theridiids and orb-weavers. Similarities in dragline attachment patterns are thus expected in *A. baeza* despite the differences in web design.

MATERIAL & METHODS

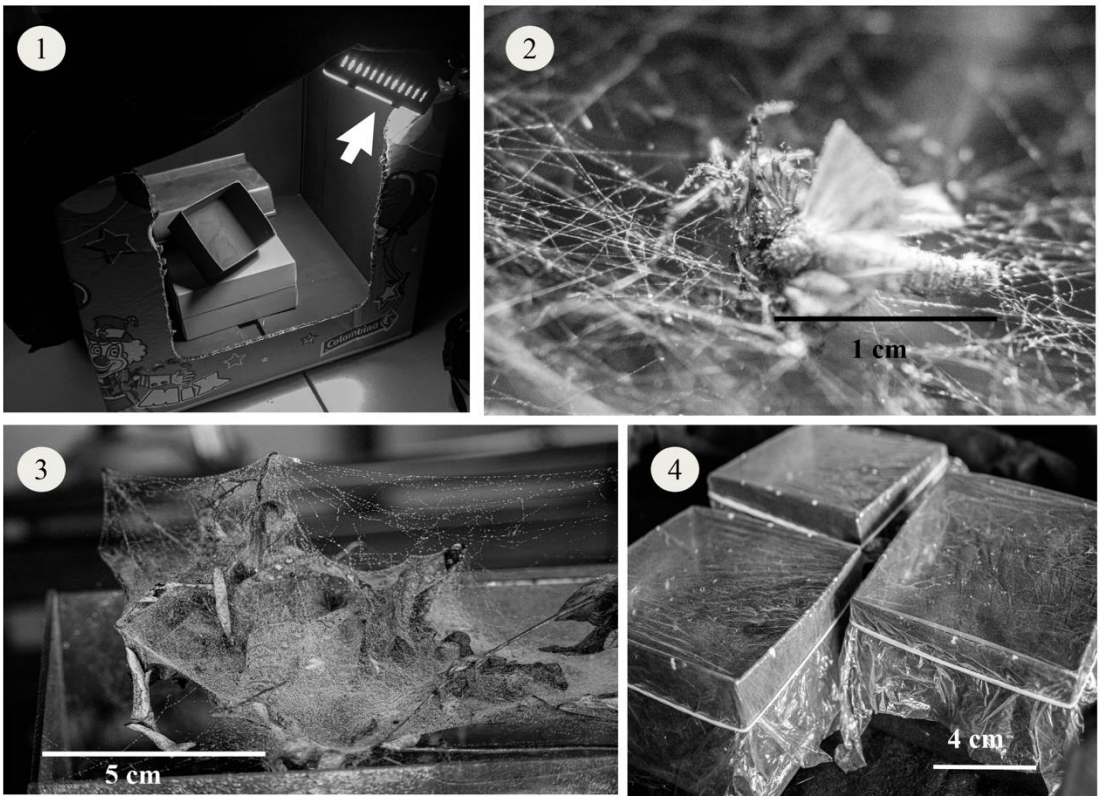
I collected colonies of *A. baeza* from January 2016 to January 2019 in Sacramento de Barva, Heredia, Costa Rica (10°5′59″N, 84°7′5″W) at 2040 m of elevation. Colonies were found on the distal extreme of branches on several bushes and trees. Most colonies were at about 3 m above the ground, and were collected from branches and placed in plastic bags for transport to the lab. Colonies contained several adults per colony and had between 10 to 60 spiders, sometimes with more than one adult. All observations were made in captivity in Heredia, Costa Rica.

Observations.— Behavioral observations were made between 18:00 and 24:00 h. Some night observations were made using dim light from a Neewer CN-160 LED light lamp not directly oriented at the spiders. Construction behavior was recorded using the infrared "night shot" option of a SONY TRV 50 digital video camera. This recording method has both strengths and limitations (Eberhard & Hazzi 2017). A strength is that the illumination glinted off lines that the spider was producing but a limitation was that only those lines that were at favorable angles to the illumination were visible. Construction events were recorded a total of 20 min every day. I recorded a total of 300 min in the colony set up and 220 min in the box set up.

Experimental set ups.—I had two types of set ups to observe dragline attachment patterns and leg movements. One was cardboard boxes 5.4 X 8 X 8.5 cm, painted with black BBQ enamel paint on the inside to create better contrast of the lines laid by the spider (Figs. 1 – 4). I placed 17 individual spiders, each in one box and let them move around for two minutes and when they tried to escape, I covered the box with tightly stretched wrapping plastic. They were fed one *Drosophila* every two days.

The second type of set up was in the spider colonies, a more familiar environment for the spiders. I observed construction in 14 colonies. I left them intact in their original substrate but maintained on 3D wire structures 20 X 19 X 29 cm or placed them in rectangular fish tanks with no cover where air could flow through them, with the precaution of maintaining the original orientation with respect to gravity. Colonies were fed with *Musca* spp. or medium size nocturnal moths (Noctuidae) (Figs.1 – 4).

In both cases, the spiders selected for observation were advanced juveniles, two instars prior to maturity (females and males are easily differentiated for the thickened pedipalps of the male), and adults of both sexes. To avoid the use of the less specific “it”, I will refer to the spiders according to their sex, “her legs”, “his legs”, etc.



Figs. 1 – 4: Set ups for observation and spiders used during the investigation. 1) Video recording set, black cardboard box illuminated with a fixed LED light, indicated with a white arrow; boxes where placed at different angles. 2) Adult *Anelosimus baeza* (Theridiidae) feeding on a moth. 3) Collected colony placed in the highest part of a fish tank holded by a wire structure. 4) Experimental boxes where the spiders built their silk structures, and construction behavior was recorded in the evening.

Behavioral patterns. — In both set ups previously described, I made video recordings of leg movements during silk thread attachment in slow motion. To test whether the web design affected thread attachment behavior I compared *A. baeza* with other spiders. Definitions of the behavioral patterns used for comparisons are in Table 1 in the Appendix.

Glossary.— Due to the inconsistent terminology in the literature of web construction behavior (Eberhard & Hazzi 2017), and in order to provide precise descriptions and abbreviations of observations, a list of important definitions for this investigation is presented in Table 2 and illustrated in the Appendix.

RESULTS

Of the 10 behaviors selected in *A. baeza* for comparisons with other groups (Table 1), seven were present in orb-weaver orbicularians (Table 3). Break and reel was not observed in the recordings of *A. baeza.* This behavior has been previously described in other theridiids while in early stages of construction of the web (Jörger & Eberhard 2006). When compared with *Acharanea tesselata* (Theridiidae) the two main differences were the absence of breaking and reeling and the use of both leg IVs by *A. tesselata* "when holding a line". This happened during construction of the sheet, *A. tesselata* briefly grasped the sheet simultaneously on either side of her spinnerets and apparently pulled the sheet or at least held it *A. baeza* used always ipsilateral legs III and IV.

The only the attachment behavior observed in the box but not in the colony was the lack of use of any legs (Table 4). This might be due to it not being used during web construction, or simply that it did not happen during recording. In contrast, several behavioral patterns were only observed in the colony setting (Table 4). One was the attachment "around the corner", which was probably due to spiders in the box being unable to attach in the outer faces of the box because the plastic wrapping was a barrier. A second difference was the attachment of a non-sticky line with only a leg III was not observed in the box. This behavior was observed while the spider was filling the sheet in the colony, making several attachments in a row; in the box spiders did not build a proper sheet, suggesting that this behavior is specific to the context of filling in the sheet.

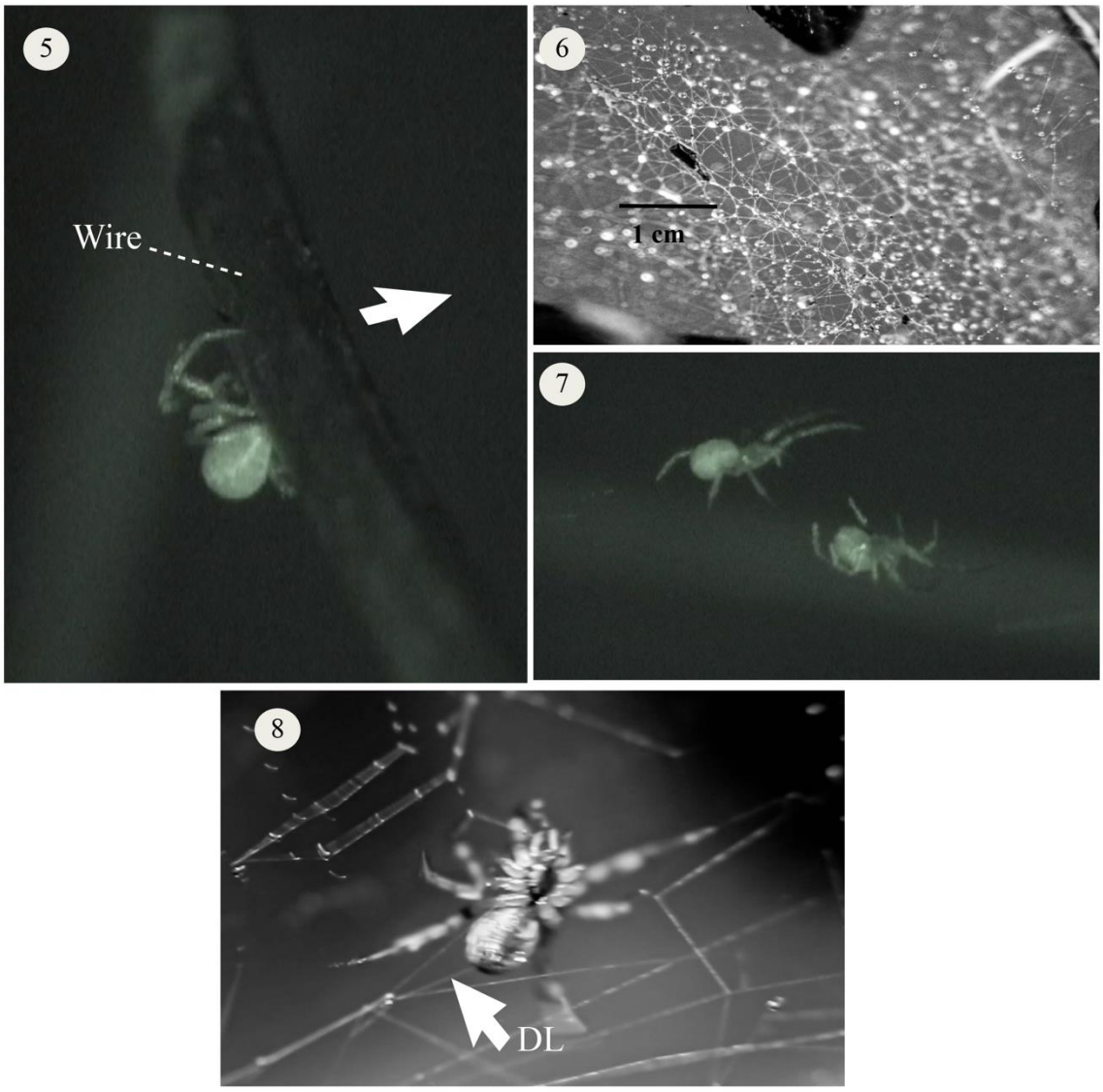
Attachment to other lines and to the substrate in the colony setting

Frame by frame analyses showed a behavior related with sheet construction, in which the spiders did continuous attachments, this while filling the sheet, presumably to make the mesh denser (Figs. 5–8). For this behavior *A. baeza* walked over the sheet producing a line from her spinnerets, but sometimes without holding it with a leg IV. While walking, the spider moved her abdomen to the left and the left leg III grabbed the produced line and then pressed her spinnerets to the sheet connecting the line. She kept walking and repeated several times before moving her abdomen to the other side using the right leg III instead to hold and connect while also walking. The spider alternated and made attachments to both sides but there was no tendency to alternate (62% of 64 consecutive attachments were to alternate sides by one spider).

When the spiders overall path was curved rather than straight, she tended to attach to the same side she was moving; for instance her overall path curved gradually to the right, she consistently attached to the right. This was different from what Eberhard & Hazzi (2017) observed in *A. castaneus*. Moving forward while at the same time moving laterally made the trajectory similar to what Jörger and Eberhard (2006) observed in *A. tesselata* but more observations are needed to determine if a pattern exists or not. Several spiders were seen doing it at the same time in the colony for an average of 10 min (Figs. 5 – 8).

Details of leg movement in the colony

The way the spider walked under a single long line (as, for instance when she was dragging a dragline to an anchor point in the colony set up) was very different from its leg movement in a three dimensional tangle. It was similar to *Caerostris darwinii* (Araneidae) (BBC 2015) and very different to what *Aglaoctenus castaneus* (Lycosidae) displayed while moving on a single long line (Eberhard & Hazzi 2017). To walk hanging from a single line, *A. baeza* hold the dragline with one



Figs 5-8: Details of attachment of lines. 5) Video image of a spider attaching “around the corner” in a wire, white arrow indicates the path the spider is going to take towards the sheet returning on the newly laid line. 6) A dense sheet of a colony on the field sprayed with water. 7) Video image of spiders cooperating filling in the sheet, one spider under the sheet, and the other spider on the top part of the sheet. 8) Dragline (DL) 28 ms after being attached in the box-tangle (BT) without using legs, spider had all legs holding a different line of the sheet then pressed her spinnerets against the line as she passed.

leg IV and to move along the single line by alternating the legs that were in front of her (Fig. 9) (in some cases the movement was not as coordinated or coordination lasted for a few seconds). When walking hanging from a line, the spider move, for example both legs II and the right leg III (R III in Fig. 9), the rest of the legs kept holding the line. Then both legs II and right leg III end up in front of both legs I and left leg III. Sequence is then repeated but the legs that move forward are the legs I and the legs that let go of the line are the ones that previously were holding the line. In contrast, when *A. baeza* walked in different planes the way it moved was by tapping or by moving in circles legs I and II to search for lines until finding them, then walking and then the rest of the legs followed moving forward.

I always observed one leg IV holding the dragline, which most probably slip through the spider tarsal claws (Eberhard & Hazzi 2017), except at times during the filling of the sheet the spider did not use a leg IV.

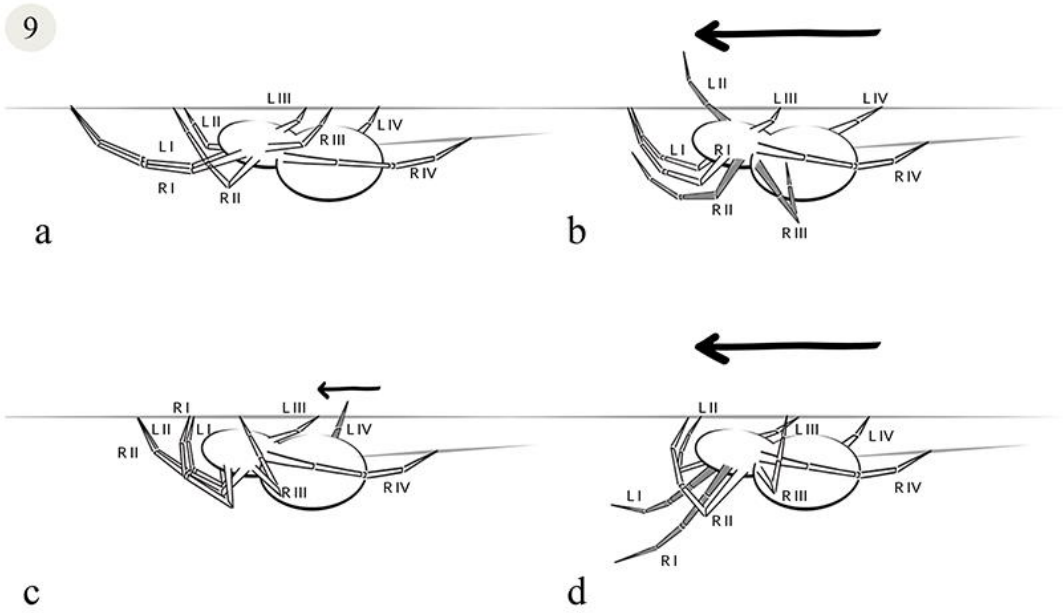


Fig 9: Schematic drawings from video images of spiders and portions of lines that glinted in the same frame (or the next one) during sheet extension in a colony (note: drawings include only a fraction of the lengths of the lines). The drawings represent the spider moving forward along a single line, illustrating leg movement patterns in a particular case. a) Initial position, all legs except a leg IV (R IV) are holding lines. R IV is holding the dragline. b) This drawing involves many movements happening at the same time. The spider moves both legs I (RI and LI) towards her body and she frees both leg II from the line. L II, R II and R III (in gray) move forward (black arrow). c) Both legs II contact the single line but their positions are different, as now legs II are in front. RIII is in front of LIII. The small black arrow indicates that L IV starts moving forward to initiate the next phase. d) As soon as L IV grabs the line again, R I and L I let go and are extended forward, R II and L II pull the body forward (black arrow) as they contract. When LI and RI contact the line all legs are again in the initial position (a). This pattern was repeated until the spider reached its destination, though in some cases the coordination was lost.

Details of leg movement in the box

One juvenile male had an unusual behavior, he elevated his abdomen vertically and walked with a loose line. Instead of using a leg IV, to hold the dragline, he maintained the abdomen in 90 degrees while walking, and occasionally used a leg IV to pull out more thread, then he attached it to substrate by putting his spinnerets against the surface.

I did not observed spiders breaking and reeling up lines along which they walked in any setting (Table 3). However, a spider in a box took lines from the box tangle to her mouth and cut them. Immediately after, she pulled an existing line she was holding with a leg III towards her abdomen and then attached a new line with her spinnerets.

DISCUSSION

Web design did not affect thread attachment behavior. In general, the other lower-level behaviors I observed were very similar in all three species of theridiids (Table 3) and very similar to those in orb-weavers. These behavioral details seem to be conserved over relatively large taxonomic groups in which the structures of finished webs vary substantially (Eberhard *et al.* 2008).

Taking the analogy from Eberhard *et al.* (2008) that compares human construction to spider webs, leg movement and attachment of lines are the bricks used to build buildings, those bricks are much less diverse than the building themselves. Theridiids and specifically *A. baeza* seem to conserve ancient construction behavior traits but have used them to evolve the web in a variety of ways (Madrigal-Brenes & Barrantes 2009). Further phylogenetic and behavior studies are needed as many of these web design traits could have evolved independently.

Consistently attaching with leg III to the same side as the spider was moving while wandering and filling the sheet is the opposite from what Eberhard & Hazzi (2017) observed in the lycosid *A. castaneus*. I did not record nor observed 'break and reel' behavior, but I cannot exclude its occurrence until detailed observations on complete web constructions were obtained. This behavior has been observed during construction of orb webs (Eberhard 1982, 1990) and has also been seen in some theridiids like *N. tesselata*, *Chrosiothes* spp. and *T. sisyphoides* (Madrigal-Brenes & Barrantes 2009). Breaking and reeling a line allows the spider to move points of attachment and to adjust the tensions on newly laid lines; Eberhard (*in press*) mentions that Freisling (1961) noted that tensions could play an important role in guiding construction behavior in the theridiid *Theridion saxatile*, especially in non-sticky lines.

Descents during exploration and web construction also occur in *Latrodectus tredecimguattatus* (Szlep 1965) S. *triangulosa* (Benjamin & Zscokke 2002). These descents may inform the spider of objects (Jörger & Eberhard 2006), in the box may have informed them of the presence or absence of lines. Descending only from the retreat is a trait of some theridiids (Madrigal-Brenes & Barrantes 2009), but not the case here, which raises the question if *A. baeza* descends or not from the retreat as it is not clear if one exists in the natural web design.

The stereotyped movement of waking under a single thread while holding with a leg IV a produced line seems to be a preserved trait (Eberhard in press). This might suggest that stereotyped

leg movements led to more diverse movements in theridiids, useful when walking in a three-dimensional web (Eberhard *et al.* 2008). *A. baeza* has several other behaviors that are shared with other theridiids like holding the dragline with one leg IV, turn back onto non sticky line just attached, walking on the substrate and attaching lines to the substrate are behaviors widespread within theridiids and orb-weavers (Eberhard in press).

In both species an erratic wandering movement is described when filling the sheet (Jörger & Eberhard 2006, Madrigal-Brenes & Barrantes 2009). The difference is that I observed several spiders cooperating filling in the sheet (Agnarsson 2006, Bilde & Lubin 2011). Attachment of anchor lines to far side of objects makes the attachments more secure (Madrigal-Brenes & Barrantes 2009). In colonies, I observed spiders attaching anchor lines to the far side of wires just like Jörger & Eberhard (2006) described in *A. tesselata*.

A. baeza showed some similarities in dragline attachment patterns with orb-weavers like attachment around the corner, stereotyped walk under single lines, turn back onto newly laid lines and the occasional ipsilateral holding of attachment lines with legs III and IV (Eberhard in press). Is not surprising that the similarities in dragline attachment patterns with theridiids were of those not present in orb-weavers like the wandering movement of filling the sheet and no holding the dragline with any leg while attaching to substrate (Benjamin & Zschockke 2003, Jörger & Eberhard 2006, Madrigal-Brenes & Barrantes 2009). I also described two new dragline behavior patterns both related to sheet construction. *A. baeza* shows a combination of dexterity (necessary in orb-weavers) and flexibility (a trait observed in web design of theridiids) (Eberhard, Barrantes & Madrigal-Brenes 2008) in dragline attachment patterns that could have contributed to their specific web design.

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LITERATURE CITED

Agnarsson, I. 2006. A revision of the New World eximius lineage of *Anelosimus* (Araneae, Theridiidae) and a phylogenetic analysis using worldwide exemplars. Zoological Journal of the Linnean Society 146: 453–593.

Agnarsson, I. & J. A. Coddington. 2007. Notes on web and web plasticity and description of the male of Achaearanea hieroglyphica (Melo-Leitão) (Araneae, Theridiidae). The Journal of Arachnology 34: 638-641.

BBC. 2015 October 30. Amazing Darwin's Bark spider spins a massive web-The Hunt: Episode 1 preview-BBC One. Retrieved from https://www.youtube.com/watch?v=gSwvH6YhqIM&t=69s

Benjamin, S. P. & S. Zschokke. 2002. Untangling the Tangle-Web: Web Construction Behavior of the Comb-Footed Spider 15: 791–809.

Benjamin, S. P. & S. Zschokke. 2003. Webs of theridiid spiders: Construction, structure and evolution. Biological Journal of the Linnean Society, 78: 293–305.

Benjamin, S. P. & S. Zschokke. 2004. Homology, behaviour and spider webs: web construction behavior of *Linyphia hortensis* and *L. triangularis* (Araneae: Linyphidae) and its evolutionary significance. J. Evol. Biol. 17: 120-130.

Bilde, T. & Lubin, Y. (2011). Group living in spiders: cooperative breeding and coloniality. En Herberstein, M. (ed.), *Spider Behaviour Flexibility and Versatility* (pp. 275-306). Estados Unidos.

Eberhard, W. G. In Press. Spider Webs: Function, Behavior and Evolution. University of Chicago Press.

Eberhard, W. G. 1976. The webs of newly emerged *Uluborus diversus* and of a male *Uluborus* sp. (Aranae: Uloboridae). Journal of Arachnology 4: 201–206.

Eberhard, W. G. 1981. The single line web of *Phoroncidia studo* Levi (Araneae: Theridiidae): A prey attractant?. The Journal of Arachnology 9: 229–232.

Eberhard, W. G. 1982. Behavioral Characters for the Higher Classification of Orb-weaving Spiders. Evolution 36: 1067–1095.

Eberhard, W.G. 1990. Function and phylogeny of spider webs. Annual Review of Ecology and Systematics 21: 341–372.

Eberhard, W. G. 1992. Web construction behavior of *Modisimus* sp. (Aranae, Pholcidae). Journal of Arachnology 20: 25–34.

Eberhard, W. G. 2018. Modular patterns in behavioural evolution: webs derived from orbs. Behaviour 155: 531–566.

Eberhard, W. G., I. Agnarsson & H. W. Levi. 2008. Web forms and the phylogeny of theridiid spiders (Araneae: Theridiidae): Chaos from order. Systematics and Biodiversity 6: 415–475.

Eberhard, W. G., G. Barrantes & R. Madrigal-Brenes. 2008. Vestiges of an orb-weaving ancestor? The "biogenetic law" and the ontogenic changes in the webs and building behavior of the black widow spider Latrodectus geometricus (Araneae Theridiidae). ethology Ecology & Evolution 20: 211–244.

Eberhard, W. G. & N. A. Hazzi. 2013. Web construction of *Linothele macrothelifera* (Araneae: Dipluridae). The Journal of Arachnology 41: 70–75.

Eberhard, W. G. & N. A. Hazzi. 2017. Web building and prey wrapping behavior of *Aglaoctenus castaneus* (Araneae: Lycosidae: Sosippinae). Journal of Arachnology 45: 177–197.

Jörger, K. M. & W. G. Eberhard. 2006. Web construction and modification by *Achaearanea*

tesselata (Araneae, Theridiidae). Journal of Arachnology 34: 511–523.

Lamoral, B.H. 1968. On the nest and web structure of *Latrodectus*. South Africa, and some observations on body colouration of *Latrodectus geometricus* (Aranae, Theridiidae). Annals of the Natal Musseum, Pietermaritzburg 20: 1-14.

Madrigal-Brenes, R. & G. Barrantes. 2009. Construction and function of the web of *Tidarren sisyphoides* (Araneae: Theridiidae). Journal of Arachnology 37: 306–311.

Rao, D. & A. Aceves-Aparicio. 2012. Notes on the ecology and behavior of a subsocial spider *Anelosimus baeza* (Araneae : Theridiidae). American Arachnological Society 40: 325–331.

Ratanaswasd, P., W. Dodd, K. Kawamura & C. Noelle. 2005. Modular behavior control for a cognitive robot. 12th Int'l Conf. on Advanced Robotics (ICAR). July 18–20.

Rojas, A. 2011. Sheet-web construction by Melpomene sp. (Araneae: Agelenidae). The Journal of Arachnology 39: 189-193.

Saffre, F., A. C. Mailleux & J. L. Deneubourg. 1999. Dragline Attachment Pattern in the Neotropical Social Spider *Anelosimus eximius* (Aranae: Theridiidae). Journal of Insect Behavior 12: 277–288.

Szlep, R. 1965. The web-spinning process and web-structure of *Latrodectus tredecimguattatus*, *L. pallidus* and *L. revivensis*. Proceedings of the Zoological Society of London 145: 75–89.

Vollrath, F. 1992. Analysis and interpretation of orb spider exploration and web-building behavior. Advances in the study of behavior. 21: 47–99.

Vollrath, F. & P. Selden. 2007. The Role of Behavior in the Evolution of Spiders, Silks, and Webs. *Annual Review of Ecology, Evolution, and Syste*matics 38: 819–46.

Appendix

Table 1. Definitions of behavioral patterns that I looked for during construction in colonies and boxes.

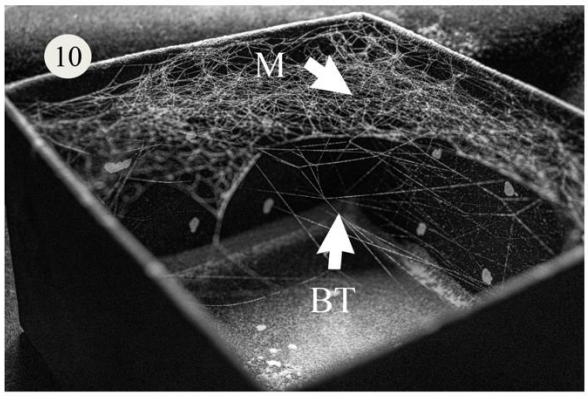
Behavioral Patterns	Description	Reference
Comparable with other groups		
Attachment "around the corner" to substrate	Attachment of anchor lines by the spider to the far side of objects	Jörger & Eberhard 2006
Exploratory descents	Descents made during exploration and construction that probably inform the spiders of presence of objects below to which they can attach or that they need to avoid	Jörger & Eberhard 2006
Hold dragline with leg IV while: walking, attaching to substrate and attaching to line	Spider holds the line with one leg IV while performing those behaviors.	Jörger & Eberhard 2006
Break and reel	Moving by cutting and reeling the previously laid dragline while constructing a new thread	Benjamin & Zschokke 2003
Legs that hold line to which attach a non-sticky line and a sticky line	Which legs attach such types of lines	Eberhard *pers. conv.*
Walk under lines holding the dragline with a leg IV and using legs on both sides	Spider walks under newly laid lines.	Eberhard in press; Eberhard & Hazzi 2017
Turn back onto a new non-sticky line just after having attached it to another line or to an object	Using a just attached line to return toward the direction from which the spider had come.	Eberhard & Hazzi 2017

Additional observations not compared with other groups		
Hold of attachment line with only a leg III	Observed in consecutive attachments, no other leg was used to attach line	Personal observations
Attachment of dragline without use of legs	Spider attaches its dragline line by pressing her spinnerets against a surface but without using any legs	Personal observations

Table 2. Definitions of important concepts. Taken from literature and others based on observations and adapted for this investigation.

Concept (abbreviation)	Definition	Reference
Web	Group of lines spunned by a spider, specifically designed for the survival and/or reproduction of the individual that spun it. Such design has a biological purpose that has suffered natural selection in the past.	Eberhard, W. G. *pers. comm*, Vollrath 1992
Construction behavior	Typically refers in the literature to the construction of the web. The term here is used in its simplest form, meaning, the response by a spider to build a structure with its silk.	Eberhard 1976, Benjamin & Zschokke 2002
Lower level behavioral patterns	Refers to patterns observed while the spider laid lines (e.g., position of legs at the moment of attachment)	Eberhard in press
Box Construction (BC)	Structure built by the spider during the captivity experiments, in a cardboard box. Structure built allowed the spiders to rest, interact, capture prey, feed and initiate courtship. No copulation was observed.	Briceño-Aguilar, observations
Sheet (S)	A tightly meshed silk structure built by the spider to capture prey.	Eberhard & Hazzi 2017
Box-Mesh (BM)	Irregular interconnected lines of silk, in this case horizontal in the top part of the box.	Briceño-Aguilar, observations
Tangle (T)	Irregular accumulation of threads above the sheet, possibly to knock down prey.	Agnarsson 2006
Box-Tangle (BT)	Tridimensional irregular accumulation of threads in a box.	Briceño-Aguilar, observations

Anchor lines (AL)	Lines used by the spider to support and extend parts of the web like the sheet or tangle, usually to substrate in the vicinity.	Jörger & Eberhard 2006
Dragline (DL)	Line of silk (presumably from the ampullate glands) produced by the spider as she moved, usually held with one leg IV	Jörger & Eberhard 2006
Lines/Thread	Silk used and laid by the spider to make webs and structures	Vollrath & Selden 2007
Colony	A tridimensional basket type web where a related group of subsocial spiders live for at least a portion of their lifespan.	Lubin & Bilde 2007
Orbicularians	Includes araneoid and deinopid families. May be paraphyletic.	Eberhard & Hazzi 2017
Exploration	Response by spider to a new space, moves around to get familiar to it, uses information for further orientation. Ends when chooses a settle point.	Vollrath 1992
Filling of the sheet	Process of filling the small spaces in the sheet	Jörger & Eberhard 2006
Extension of the sheet and extension of the tangle	Connect anchor lines to either sheet or tangle to substrate on the vicinity, thus extending the area covered by the web	Jörger & Eberhard 2006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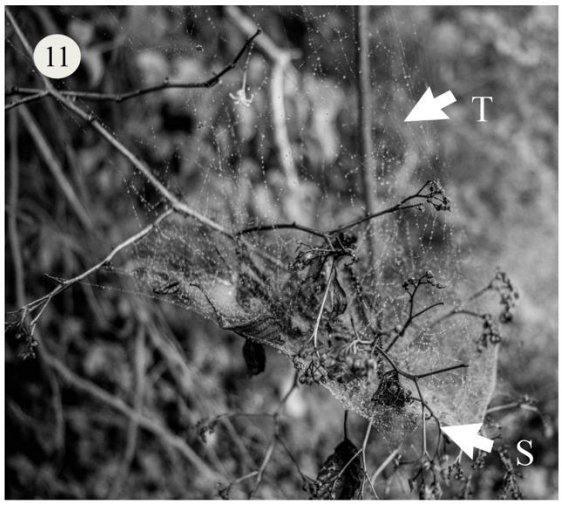
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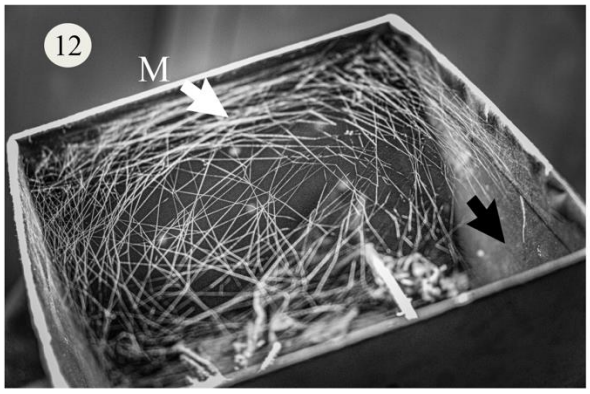
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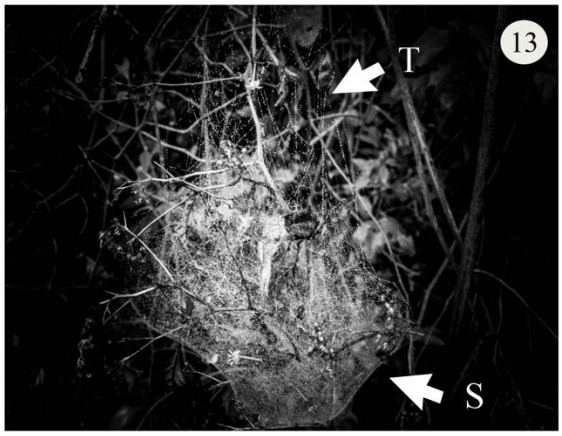
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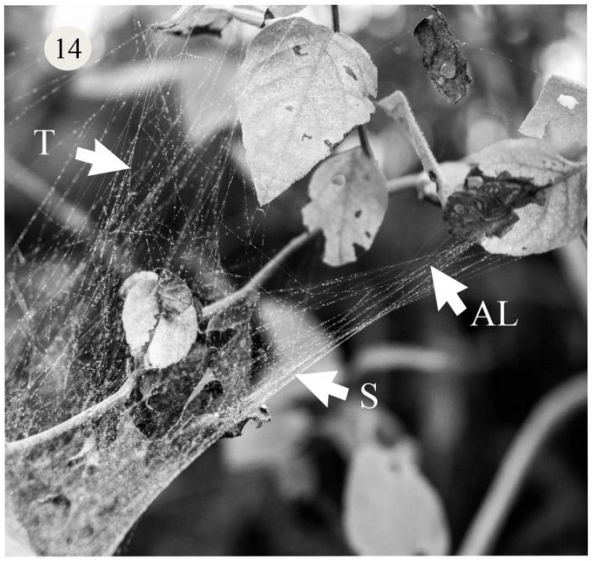
14
T
AL
S


15

Figs. 10– 15. Companion figures of the glossary as per Table 2. 10) Silk construction of a spider in a box covered with talcum powder for better contrast. Mesh (M) covers top part of the box and below is the irregular box tangle. 11) *A. baeza* colony seen from above in the field sprayed with water for contrast with dead leaves incorporated into it. Tangle (T) in the top part and sheet (S) in the lower part of the web. 12) Detail of the mesh (M) as it extends all over the top part of the box. A few lines (black arrow) appear missing but is due the camera not being able of capturing all lines in that angle. It was not covered with talcum nor water. It was photographed only with a single fixed LED light. 13) Typical basket web form of an *A. baeza* colony in the field. Tangle (T) in the top part and sheet (S) in the lower part of the web 14) Detail of different parts of a *A. baeza* colony in the field. Tangle (T) in the top part of the web, anchor lines (AL) attached at leaves and the sheet (S) extending from it. 15) Detail of Tangle anchor lines sprayed with water attached to leaves.

Table 3. Patterns observed directly in the building behavior of non-orb building spiders. Modified from Eberhard *in press* for the purposes of this study. (* For behaviors observed in colonies in *A. baeza*) (‘’ For behaviors observed in boxes in *A. baeza*).

Taxon	Attach “around corner” to substrate (*)	Exploratory descents (*)(‘’)	Hold dragline with IV while: walking (*)(‘’)	Hold dragline with IV while: Att. to substrate (*)(‘’)	Hold dragline with IV while: Att. to line (*)(‘’)	Break and reel	Legs that hold line to which att. Non-sticky (*)	Legs that hold line to which att. Sticky	Walk under lines(*)	Turn back onto n-s line just attached (*)	Reference
Mygalomorpha											
Dipluridae											
Linothele macrothelifera	No	No[1]	No	No	No	No	None	N.A	No[1]	No	Eberhard & Hazzi 2013
Araneomorpha											
Non-orbicularia											
Pholcidae											
Modisimus guatuso	?	Yes	?	Yes	?	No	III	None	Yes	Yes[2]	Eberhard 1992, WE
Eresidae											
Stegodyphus sp.	?	?	?	?	?	?	ipIII&IV[3]	?	Yes	?	WE
Agelenidae											
Melpomene sp.	?	No	No	No	No	No	No	No	No	No	Rojas 2011
Lycosidae											
Aglaoctenus castaneus	?	Yes	No	Occasionally	No	No	III[4]	N.A	Yes/No	Yes	Eberhard & Hazzi 2017
Orbicularia											
Theridiidae											
Latrodectus geometricus	?	?	Yes/No[5]	Yes	Yes	Yes	ipIII&IV[6]	III&IV	Yes	Yes	Lamoral 1968, Eberhard et. al 2008
Achaearanea tesselata	Yes	Yes	Yes	Yes	Yes[7]	Yes[8]	III&IV/IV&IV[9]	N.A	Yes	Yes	Jörger & Eberhard 2006
Anelosimus baeza	**Yes**	**Yes**	**Yes/No[10]**	**Yes/No[10]**	**Yes/No[11]**	**?[12]**	**ipIII&IV[12]**	**N.A**	**Yes**	**Yes**	**Eduardo Briceño-Aguilar**
Linyphiidae											
Lynyphia hortensis	?	?	Yes	?	?	No[13]	?	?	Yes	Yes/No[14]	Benjamin & Zschokke 2004
Orb-weaving orbicularians	Yes	Yes	Yes	Yes	Yes	Yes	ipIII&IV	ipIII&IV	Yes	Yes	Eberhard 1982

[1]Site were built (on the surface of leaf litter) was not appropriate for this type of behavior.

[2]During construction of the skeleton web only; did not occur during filling of skeleton web or while laying sticky lines (in the latter case, the spider turned back repeatedly at the edge of the web, but did not return along the same line that she had been laying)

[3]Ocassionally only III

[4]Very rarely ipsilateral

[5]The exception is during production of sticky lines, when no leg touches the newly produced line; leg IV also does not hold the line just after the spider begins a descent to lay a new gumfoot line

[6]With both legs III and only IV when initiating and finishing gumfoot lines, and also in central area

[7]Not always; apparently, late in construction of the sheet, the spider sometimes grasps the sheet with both IV and does not hold the dragline

[8]During early stages ("exploration") only

[9]When building sheet, the spider does not hold line to which attachment is being made with any legs

[10]The exception to use of legs a one juvenile male (2 instars before maturity) walking around with his abdomen elevated at the edge of the box and then contacting the substrate directly with his spinnerets with no legs nearby.

[11] The exception to use leg IV occurred in the box when the spider was connecting lines in the tangle and the tangle was very dense; the new lines were always short, all legs were holding lines and it might not be necessary to hold the dragline, as attachment was easily done by just a movement of the abdomen.

[12]When filling the sheet with consecutive attachments, only uses a III, side depends on direction of the abdomen, if it moves to the left uses left leg III to attach, and so forth.

[13]Failure of some authors to see this behavior in theridiids, where it occurs (see Eberhard et al. 2008) makes this characterization uncertain.

[14]Contradictory accounts were given on p.123 in the first and third paragraph of Benjamin & Zschokke (2004). Perhaps the "yes" refers to non-sticky lines, and the "no" to the sticky lines.

Table 4. Summary of behavioral observations in *A. baeza* during construction. The context (construction stage) and the location (set up) of where it was observed is given.

Behavioral observations	Set up	Context in which it was observed
Movement of legs		
Exploratory descents[1]	-Box -Colony	-Exploration -Extension of the tangle
Hold dragline with leg IV while walking[1]	-Box -Colony	-Exploration / Retreat construction/ Adding lines to the tangle-mesh -Extension of the sheet / Extension of the tangle
Legs (ip III & IV) that hold line to which attach a non-sticky line[1]	-Box -Colony	-Construction of the retreat / Adding lines to the tangle-mesh -Filling of the sheet
Legs that hold line to which attach a sticky line[1]	-N.A	-N.A
Walk under lines[1]	-Box -Colony	-Adding lines to the Tangle-Box -On newly attached lines for extension of the sheet
Turn back onto non-sticky line just attached[1]	-Colony	-Extension of the tangle
Break and reel[1]	-N.A	-N.A[3]
Attachment to other lines and to the substrate		
Attachment "around the corner" to substrate[1]	-Colony	-Extension of the sheet / Extension of the tangle
Attachment of non-sticky line with only leg III[2]	-Colony	-Filling of the sheet[4]
Hold dragline with leg IV while attaching to substrate[1] or line[1]	-Box -Colony	-Exploration (to substrate)/ Adding lines to the tangle-mesh (to line)[5] -Extension of the tangle (to line)
No holding the dragline and attaching to substrate or line[2]	-Box	-Exploration[6] (to substrate) / Adding lines to the tangle-mesh

[1]Behaviors used for comparisons
[2]Additional Behaviors observed
[3]Breaking of lines with mouth was observed in the box set up.
[4]Occasional use of a leg IV to pull more thread
[5]One female observed switching leg IV that hold the dragline before attaching it.
[6]Male with elevated (vertically) abdomen while walking